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A STRAIN OF THE ALFALFA-MOSAIC VIRUS CAUSING VINE AND TUBER NECROSIS IN POTATO

JOHN W. OSWALD[1]

(Accepted for publication March 27, 1950)

In 1946 attention was called to a devastating disease affecting potatoes of the White Rose variety growing near Stockton, California. The most striking symptom was an internal necrotic browning of the main shoots, petioles, and leaf veins. The vines were stunted, the top leaves rugose and tightly rolled upward but without visible mottle. As the disease progressed, leaves and whole shoots were killed and in severe cases the vines collapsed completely. Tuber set was light and most tubers from such vines had internal necrosis. Diseased vines were most common along those margins of the field adjacent to an alfalfa planting. In the same field the potato-calico disease caused by a strain of the alfalfa-mosaic virus (2) was prevalent and occurred in some plants that had the severe necrotic condition. The symptoms of the necrotic disease were so strikingly different from those of ordinary potato calico that such viruses as yellow dwarf, spotted wilt, Canada streak, and Virus Y were at first suspected as more likely causal agents.

The author's preliminary report on this disease (9) indicated it was attributable to a virus easily transmissible by mechanical means. Subsequent investigations on host range, virus properties, and cross protection have led to the conclusion that the necrotic disease is caused by a strain of the alfalfa-mosaic virus, hereafter referred to as the tuber-necrosis virus.

White Rose is the principal potato variety grown in California. Since its discovery in the Stockton area in 1946, the tuber-necrosis disease has been found occasionally on White Rose in Kern County, the State's principal early potato section, and has recently been observed in the field in the Pontiac variety. In all cases diseased vines have been in fields adjacent to alfalfa plantings.

Previous references to the alfalfa-mosaic virus in connection with potatoes have been those dealing with the potato-calico disease. In the descriptions of its effects upon potatoes, no mention has been made of any vine or tuber necrosis.

Potato calico was first described in 1922 by Hungerford (4), who considered it a genetic aberration rather than an infectious disease. Porter (11, 12) was the first to demonstrate clearly that it was infectious and transmissible by juice and aphid inoculation. His studies showed tomato, pepper, Datura, eggplant, and petunia to be hosts of the virus. During

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his investigations he observed that potato calico occurred most commonly in fields growing in the proximity of alfalfa but he was unable to confirm by inoculation that alfalfa was a natural host plant.

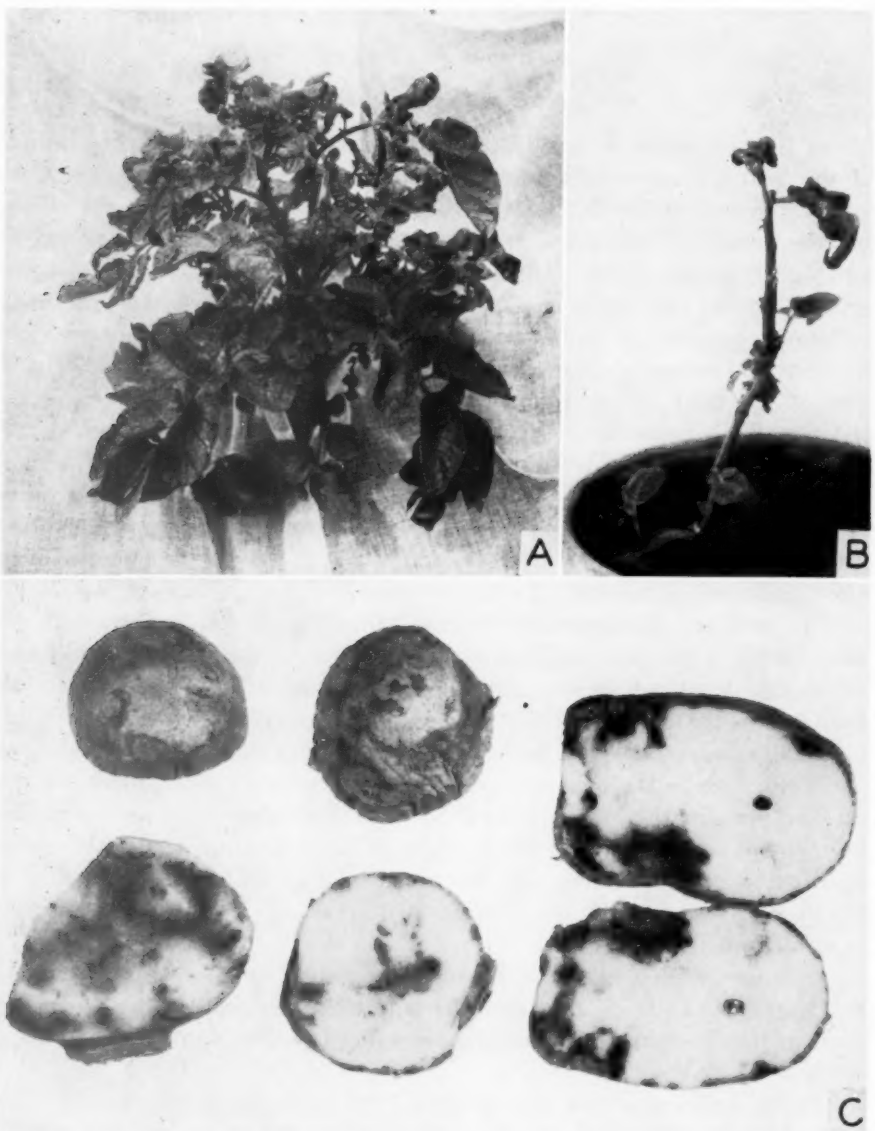

FIG. 1. White Rose variety of potato naturally infected with the tuber-necrosis virus. A. Vine showing current-season infection. B. Vine grown from an infected tuber showing tuber-borne symptoms. C. Necrotic tubers produced on badly diseased vines.

Dykstra (3), in his studies on the yellow mosaics of potatoes, concluded on the basis of symptoms, virus properties, and protective cross inoculations that the potato-calico virus was distinct from the aucuba-mosaic, tuber-

blotch, and Canada-streak viruses. He investigated the virus properties and host range of calico but made no reference to any relationship to the alfalfa-mosaic virus.

In 1940 Black and Price (2) found that alfalfa mosaic and potato calico were caused by closely related but not identical viruses. The potato-calico virus caused more severe symptoms on potato and induced a milder reaction in *Nicotiana glutinosa* L., cowpea, and bean than did the alfalfa-mosaic virus. Cross inoculation tests showed that tobacco plants infected with the potato-calico virus were refractory to infection with the alfalfa-mosaic virus. Black and Price considered the potato-calico virus as a strain of alfalfa-mosaic virus and gave it the trinomial *Marmor medicaginis* H. var. *solani* Black and Price.

Recently Berkeley (1) has described a strain of alfalfa-mosaic virus in pepper, *M. medicaginis* H. var. *capsici* Berkeley, which he compared with common alfalfa-mosaic virus and potato-calico virus. His results also confirm the close relationship of the calico virus to the alfalfa-mosaic group. He differentiates the calico virus from the other two strains on its inability to attack cucumber and on the slightly different symptoms it induced on *Nicotiana rustica* L., *N. glutinosa*, and pepper.

SYMPTOMS OF THE TUBER-NECROSIS VIRUS ON POTATO

Current-season infection in the field is marked first by rugosity and necrosis of the top leaves (Fig. 1, A). The leaflets fold upward along the main veins with the tips curling downward. More striking is the internal necrotic browning that affects the majority of the veins and petioles. The cortical, vascular, and pith tissues turn a rusty brown color, but the epidermal layers are unaffected. Such veins and petioles remain turgid until the entire leaf dies. This necrosis is distinct from the blackening, streaking, and collapse of veins caused by virus Y (rugose mosaic). The necrosis extends into the branches and main shoots and is most severe at the nodes. The cortical and vascular tissues of split stems are brown but firm; the pith may be flecked with brown necrotic blotches.

As the disease progresses, the leaves become necrotic along their margins and eventually collapse and die. Often the virus may affect but one shoot of the plant or but one side of one shoot. Occasionally the tip may be killed before lower parts of the vine are affected (Fig. 3, D). A yellow blotching of the leaves that frequently develops in the field, along with these necrotic symptoms, is attributed to the calico virus with which the tuber-necrosis virus is often closely associated.

The symptoms resulting from mechanical inoculation are similar to but usually more severe than those occurring naturally in the field (Fig. 2, A). On the inoculated leaves veinal discoloration occurs as the first visible symptom 5 to 7 days after inoculation, followed shortly by the development of black necrotic areas along the margins of the leaflets and yellowing of tips. Within 10 to 12 days the veins of the inoculated leaves are totally

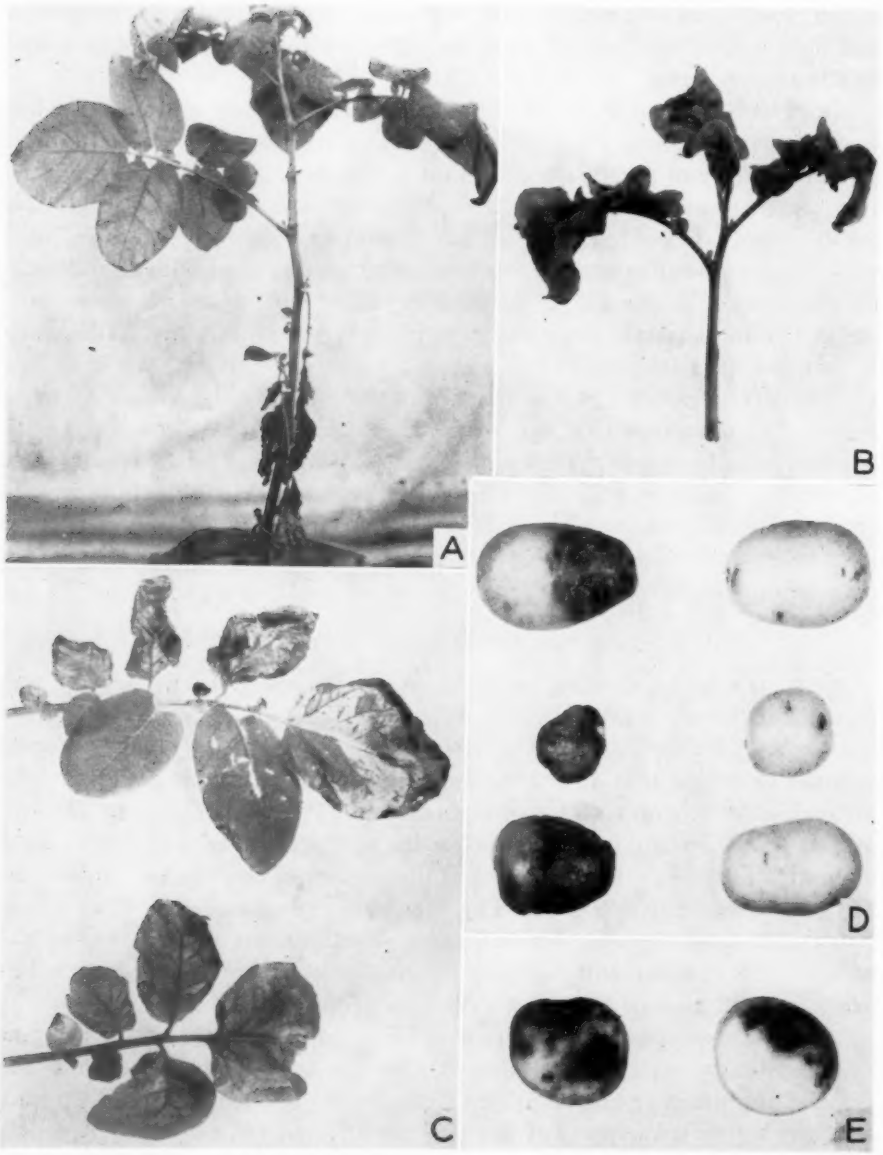

FIG. 2. Symptoms in White Rose variety of potato artificially inoculated with the tuber-necrosis virus. A. Vine 2½ weeks after inoculation. Note internal discoloration in shoots and veins. B. Rugosity of leaves in growing tip of infected vine. C. Rubbed leaves 2 weeks after inoculation. D. Left, three tubers produced by necrotic vine that was inoculated directly from a mosaic-infected alfalfa plant; right, three check tubers. E. Cross section of two of the infected tubers illustrated in D.

necrotic, as are the petioles. Inoculated leaves turn yellow in large areas and the leaflets become dry along the margins (Fig. 2, C). At this stage the virus is systemic, and occasional veins in the youngest leaves are necrotic.

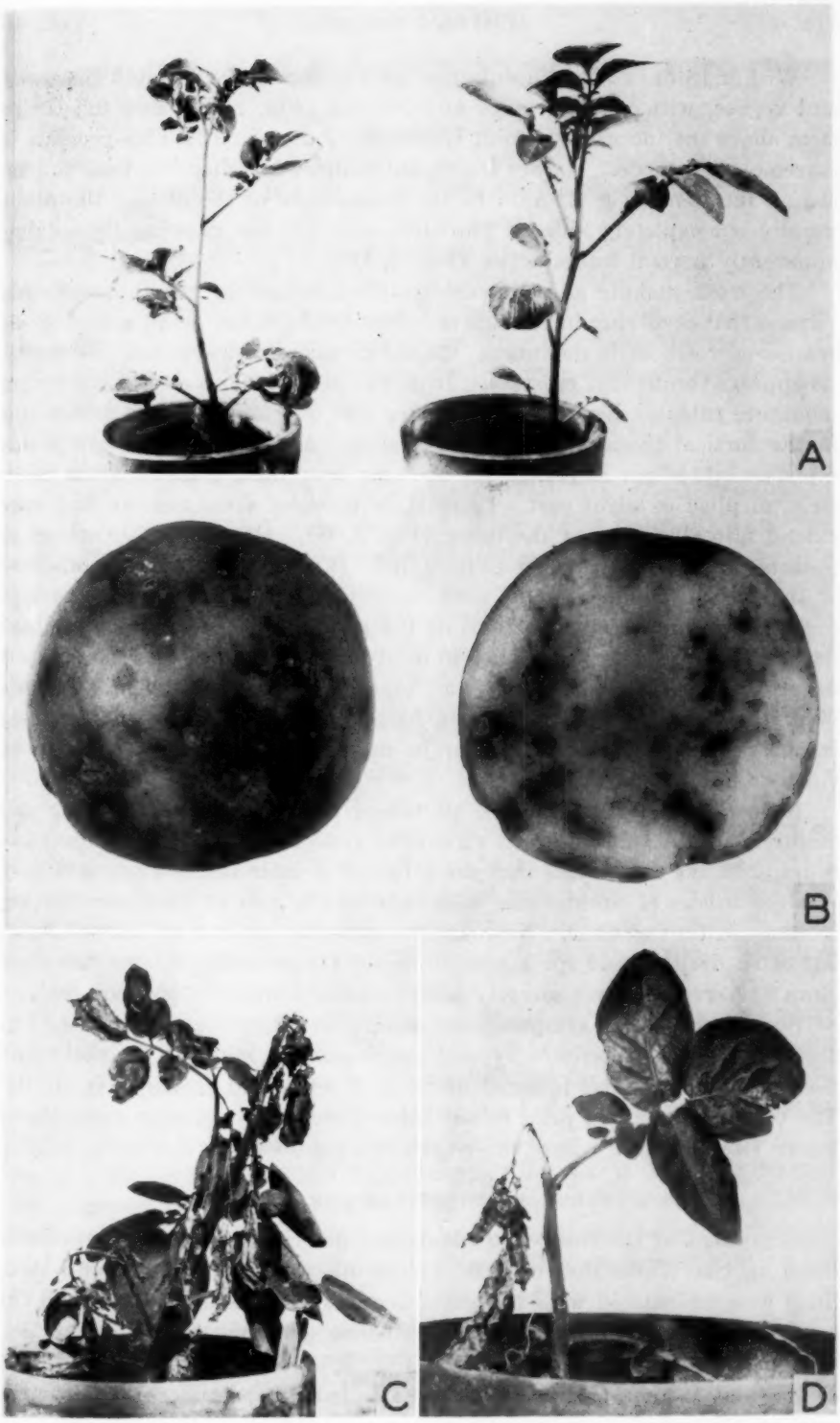

FIG. 3. Symptoms of the tuber-necrosis virus in potato. A. U. S. D. A. Seedling 41956. Left, vine grown from tuber infected with tuber-necrosis virus; right, healthy check. B. External (left) and internal (right) necrosis in a tuber of the X-immune seedling inoculated with the tuber-necrosis virus. C. Vine of Mohawk variety mechanically inoculated with tuber-necrosis virus. D. Chippewa vine infected with tuber-necrosis disease.

Within 18 days after inoculation the top leaves are severely puckered and rugose, with necrotic veins and petioles (Fig. 2, B), and the entire stem above the inoculation point is internally discolored. This necrosis is extreme at the nodes. Lower leaves are completely killed but tend to persist on the stem (Fig. 2, A). In the final stages of the disease the plant usually is completely killed. The virus may kill the growing tip leaving apparently normal leaves below (Fig. 3, D).

The most striking and unusual symptom caused by the tuber-necrosis virus is that occurring in the tubers. Just as the veins, petioles, and stems become necrotic, so do the tubers. As the virus becomes systemic, the necrosis appears throughout the vine. It occurs in the stolons and in the young immature tubers. Necrosis in the tuber first develops near the stolon end in the cortical tissues just under the skin. At this stage the symptoms resemble late-blight infection, for the skin over these necrotic areas takes on a purplish to silver cast. Later these diseased areas enlarge and may extend into all tissues of the tuber (Fig. 1, C). Often necrosis occurs as pockets throughout the tuber (Fig. 3, B). It may develop on but one side of the tuber. Frequently the necrosis occurs in rings or spirals enveloping otherwise unaffected portions of the potato. The necrotic tissues are deep brown, very dry, and corky to mealy in consistency. As the necrosis deepens the surface layers dry out, crack, and become sunken. At this point the tuber disease resembles a fungus dry rot. These sunken areas in the skin may be very irregular in pattern or may be in the form of rings or half-moons (Fig. 1, C).

Necrosis usually develops in all tubers produced on vines killed prematurely by the tuber-necrosis virus; the yield is small and the tubers are rough. In the field, vines that are infected in later stages of growth may produce tubers of normal size with only one or two of them necrotic or, in some instances, none. Necrosis is commonly present at harvest time, but often deepens and spreads in storage. Occasionally, tubers that were clean at harvest became severely necrotic after 6 weeks of storage.

Second-generation symptoms are similar to those of current season but are usually more intense. Typical symptoms are stunting, rugosity and flecking of leaves, and internal necrosis of veins and shoots (Fig. 1, B). The vine may be killed prior to any tuber formation. In most cases plants grown from diseased tubers rarely produce potatoes over 2–3 oz. in weight.

VARIETAL SUSCEPTIBILITY IN POTATO

Symptoms of the tuber-necrosis disease described above are principally those on the White Rose variety. Nine other potato varieties and seedlings were inoculated with the tuber-necrosis virus and all developed the same general current-season and tuber-borne symptoms as White Rose.

Netted Gem, Pontiac, Mohawk (Fig. 3, C), Chippewa (Fig. 3, D), Teton, and Houma all were severely affected by the tuber-necrosis virus and in all varieties necrotic tubers were produced. Sequoia and Red

Warba proved to be susceptible, but in neither variety were necrotic tubers encountered.

The X-immune U. S. D. A. seedling 41956 was tested early in the investigations and found to be highly susceptible. Inoculated leaves are blighted and as the virus becomes systemic the shoots and tubers develop necrosis (Fig. 3, B).

The tuber-necrosis virus was tested on 3-week-old potato plants grown from true seed from the cross Katahdin × B287-2. Twelve of the 15 inoculated seedlings became infected and all were dead within 3 weeks. The first evidence of infection was necrotic spotting and veinal necrosis on the inoculated leaves. The young shoots soon became internally necrotic and tips died back.

TRANSMISSION OF THE TUBER-NECROSIS VIRUS

Tuber transmission. The tuber-necrosis virus has clearly been shown to be tuber-perpetuated. Hundreds of tubers from naturally occurring diseased plants have been indexed. Approximately 80 per cent of those that grew produced necrotic vines. Wherever tuber necrosis involves eyes, the buds are killed. Thus many of the more severely affected tubers fail to yield plants. Of 129 tubers produced on vines artificially inoculated in the greenhouse, 122 or 94 per cent yielded infected plants. Non-necrotic tubers from diseased plants in most instances proved to be infected with the virus.

Mechanical transmission. Initial attempts to transmit the tuber-necrosis virus by mechanical application of expressed filtered juice were 100 per cent successful. Throughout all these investigations it has been observed that whenever a good source of virus is employed, juice inoculation is very satisfactory. In the course of experimentation, approximately 500 potato vines have been inoculated with expressed juice, with over 95 per cent success. In addition, numerous plants of other susceptible hosts have been similarly inoculated, with success.

Carborundum powder used as an abrasive is of value in increasing the percentage of infected plants. However, in one series, seven of 10 plants inoculated without the abrasive became infected.

Graft transmission. To determine whether the tuber-necrosis virus was graft-transmissible, diseased scions were grafted to 15 healthy White Rose plants in the greenhouse. In approximately 3 weeks necrosis appeared in the shoots of two vines just above and below the point of union. Later both of these plants and their tubers became typically necrotic. Two additional plants proved to be infected when their tubers were indexed.

Insect transmission. Porter (12) and Dykstra (3) demonstrated the potato aphid, *Macrosiphum solanifolii* Ashm., to be a vector of potato calico. The pea aphid, *Macrosiphum pisi* Kalt., readily transmits alfalfa mosaic (14). Kovachevski (6) showed the green-peach aphid, *Myzus persicae* Sulz., to be a vector of the disease in chilli pepper.

In the preliminary search for an insect vector only *M. persicae* was tested. Nonviruliferous colonies of the aphid were fed for a week on potato plants infected with the tuber-necrosis disease and then were transferred to healthy White Rose plants for a 4-day feeding, approximately 50 aphids to each test plant. Of 51 plants thus inoculated, 8 or 15.7 per cent became infected with the tuber-necrosis virus. The first evidence of disease was veinal necrosis and black necrotic spotting of the older leaves 16 days after inoculation. Later more severe symptoms developed similar to those induced by juice inoculation. This indicates that the green-peach aphid is a vector of the tuber-necrosis virus.

COMPARISON OF SYMPTOMS CAUSED BY THE TUBER-NECROSIS VIRUS ON VARIOUS HOSTS WITH THOSE CAUSED BY THE POTATO-CALICO VIRUS

The tuber-necrosis and potato-calico diseases often occur in the same general areas in fields. Frequently plants with brilliant calico blotching of leaves will also have tuber-necrosis symptoms in the shoots and tubers. Other plants have only calico, and still others only the necrotic symptoms. Efforts to separate the necrotic from the calico symptoms by repeated subinoculations were successful. This indicated that two viruses or strains were involved. Since field evidence pointed to a relationship between the two viruses, their effects on several different hosts were compared.

In these studies the source of the tuber-necrosis virus was a White Rose potato plant from a field near Stockton, California. The virus was passed through potato plants several times and produced only the typical tuber-necrosis disease. It was then inoculated into the X-immune seedling 41956 to eliminate virus X. These inoculated X-immune plants were the source of virus for symptom studies.

Two sources of potato-calico virus were used. One was from a potato plant growing in the above-mentioned Stockton field. From it the virus was passed through potato and produced only the yellow blotching symptoms. The virus was then inoculated into X-immune seedlings. The other source was a Netted Gem tuber grown in Idaho and this isolate likewise was passed through the X-immune seedlings. In all investigations on host symptomatology and properties the two isolates of the potato-calico virus proved to be identical.

Work by Black and Price (2) and by Berkeley (1) has established that the potato-calico virus is a strain of the alfalfa-mosaic virus. In view of this, such plants as bean, pea, pepper, tobacco, and cowpea, all known to be good diagnostic hosts for alfalfa-mosaic virus, were used in the comparative symptomatology studies of the potato-calico and tuber-necrosis viruses. All inoculations were made by the mechanical application of expressed juice to young leaves using carborundum as an abrasive. A summary of the reaction of 12 different hosts to the two viruses is in table 1. Figure 4 illustrates the effects of the tuber-necrosis virus on 10 hosts.

Potato. On potato the effects of the two viruses are easily distinguish-

TABLE 1.—*Reaction of twelve species of plants to inoculation with the tuber-necrosis and potato-calico viruses*

Host	Tuber-necrosis virus			Potato-calico virus		
	No. of plants[a]		Symptoms	No. of plants[a]		Symptoms
	Inoculated	Infected		Inoculated	Infected	
Nicotiana tabacum, var. Turkish	45	27	Systemic concentric ring patterns, striations, and oak-leaf patterns on leaves.	21	20	Systemic yellow mottling and blotching of leaves; veins remain green; symptoms fade with age.
N. rustica	38	25	Diffuse systemic yellow mottle of leaves followed by rings and striations.	18	9	Systemic yellow leaf mottle; green veinbanding; yellow blotches with green islands.
N. glutinosa	41	30	Systemic yellow blotches; rings and necrotic leaf spotting; veins cleared, rugose.	21	14	Systemic yellow leaf mottle; green veinbanding.
Pepper, var. California Wonder	100	83	Reddish-brown necrotic local lesions on rubbed leaves; systemic yellow chlorosis; dark green veinbanding.	40	36	Identical to tuber-necrosis virus.
Tomato, var. Pearson	35	0	None.	10	0	None.
Datura stramonium	43	17	Systemic yellow leaf mottle; veins cleared; green veinbanding.	32	15	Identical to tuber-necrosis virus.
Pea	12	12	Necrotic black local lesions on rubbed leaves.	12	12	Systemic yellow interveinal mottle; pin-point necrosis; streaking of veins.
Cucumber	6	6	Severe systemic yellow mottle, extreme distortion and puckering of leaves.	9	9	Identical to tuber-necrosis virus.
Celery	12	10	Systemic yellow mottle; yellow rings and green islands; later bleaching.	14	10	Identical to tuber-necrosis virus.
Cowpeas, var. Black-eye	10	10	Small (1–2 mm.) brown to black, solid local lesions on rubbed leaves.	10	10	Larger (5 mm.) irregular shaped, brick red, local lesions; red veinal necrosis.
Alfalfa	32	18[b]	Black lesions on rubbed leaves; systemic yellow rings and mottle of leaves.	35	15[b]	Similar to tuber-necrosis virus.
Bean, var. Bountiful	52	52	Local lesions on rubbed leaves; lesions small (1–2 mm.), dark brown with tan centers.	43	43	Local lesions on rubbed leaves; lesions large (4–5 mm.), irregular outline, netted in appearance and reddish brown; severe reddening and necrosis of veins of rubbed leaves.
Do, var. pink	3	2		6	6	
Do, var. Red Valentine	6	6		6	6	

[a] Total numbers of plants from two to ten different inoculation series.

[b] Only five of the alfalfa plants inoculated with the tuber-necrosis virus and seven of the plants inoculated with the potato-calico virus had alfalfa-mosaic symptoms. The symptomless plants were proven to be infected by recovery of the respective virus in bean.

able. The principal symptom of the calico virus is a brilliant yellow to white blotching of the leaves. In Hungerford's (4), Porter's (11), and Dykstra's (3) observations, this is the only symptom described, no necrosis being mentioned, but in the author's work, a necrotic spotting of the leaves and a black streaking of the shoots occasionally occur. One to 2 weeks

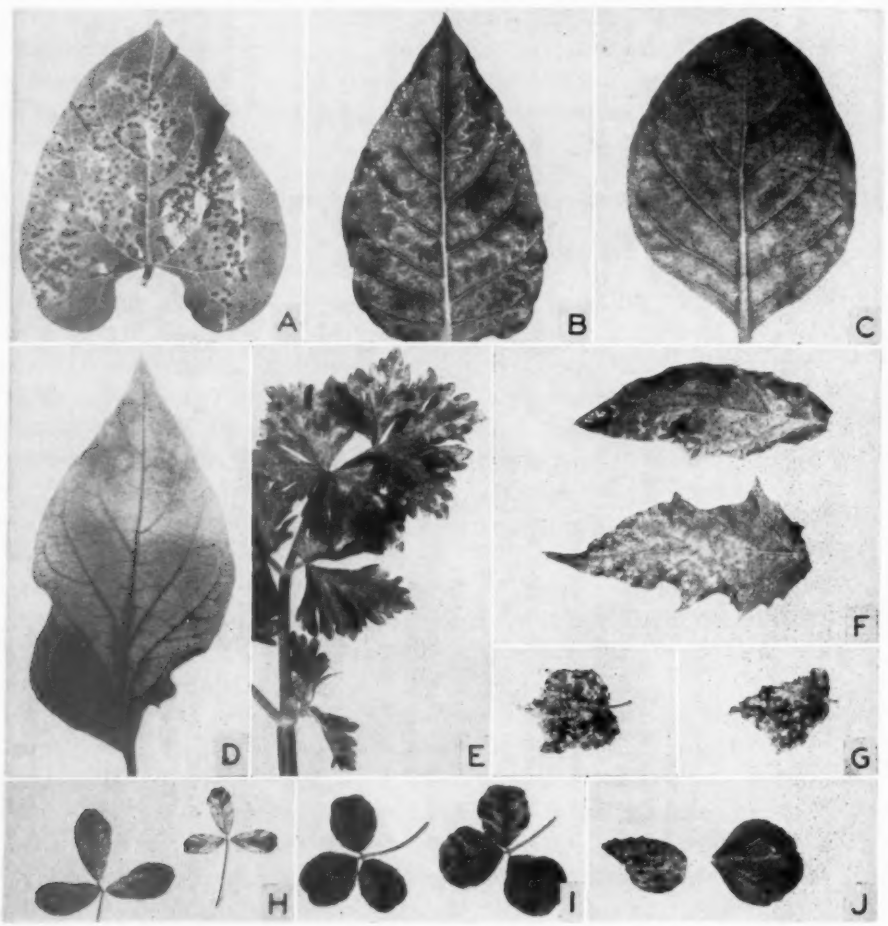

FIG. 4. Symptoms of the tuber-necrosis virus on 10 different hosts. A. Local lesions on Bountiful bean. B. Oak-leaf pattern on Turkish tobacco. C. Yellow mottle on *Nicotiana rustica*. D. Chlorosis and veinbanding on pepper. E. Yellow mottle on celery. F. Mottle and veinbanding on *Datura stramonium*. G. Severe mottling and puckering on cucumber. H. Yellow rings and mottle on alfalfa. I. Yellow streaking on Ladino clover caused by a virus recovered from Ladino clover and very similar to tuber-necrosis virus. J. Local necrotic lesions on pea.

after inoculation, black necrotic spots may appear on the rubbed leaves and more rarely on the newer growth. After 3 weeks the typical yellow blotching develops in the youngest growth. Potatoes inoculated with the calico virus just prior to or during hot weather (over 80° F.) may have only occasional black necrotic spots on the leaves and no calico symptoms. Subinoculations from such vines have shown the calico virus to be present.

No tuber necrosis attributable to the calico virus has been reported by others or observed in these investigations.

Bean. Local lesions develop on bean (variety Bountiful) within 36–48 hr. after mechanical juice inoculation with the tuber-necrosis virus. The lesions are small, 1–2 mm. in diameter, and dark brown with tan centers (Fig. 4, A). Because of the rapidity of symptom expression after inoculation, bean has been used as a test host for virus recovery in other inoculated host plants.

The local lesions induced by the calico virus are large (4–5 mm. in diameter), irregular in outline, netted, and reddish-brown. In addition veinal reddening in the inoculated leaves is a common symptom of the potato-calico virus on bean.

Occasionally both viruses become systemic, causing necrotic dieback of the tip and main stem.

Pepper. The California Wonder variety is systemically infected by both the tuber-necrosis and potato-calico viruses. The symptoms caused by the two viruses on pepper are indistinguishable. Both cause local, reddish, necrotic lesions to develop on the rubbed leaves 7 to 10 days following inoculation. Chlorosis of the upper half of the leaf and dark green veinbanding are the characteristic features of systemic infection (Fig. 4, D). Accompanying this chlorosis are roughening and wartiness of the veins. It is only with difficulty that either virus can be recovered from pepper. Practically all inoculations from pepper to bean and pepper to potato have been negative.

Tomato. In no case has either virus been successfully introduced into or recovered from this host.

Datura stramonium L. Both viruses cause systemic yellow mottles that are similar in all respects (Fig. 4, F). The veins are clear with definite dark green borders. Though the symptoms are indistinguishable in Datura, each virus upon recovery caused its typical symptoms on potato and bean.

Nicotiana tabacum L. var. *Turkish.* Both viruses produce rather severe and striking symptoms in the first month after inoculation, but the effects fade perceptibly thereafter. The tuber-necrosis virus causes lines, patterns, concentric rings, and striations on Turkish leaves. Sometimes a distinct oak-leaf pattern develops, as in figure 4, B. This is similar to Zaumeyer's (15) illustration of Turkish infection by alfalfa-mosaic virus.

The potato-calico virus symptoms are quite different on this host. Rings and line patterns are generally lacking. A yellow mottle and blotching of the leaves with green veinbanding is the principal effect.

Nicotiana glutinosa L. As in Turkish tobacco, the tuber-necrosis virus causes principally ring and line patterns on this host. In addition, necrotic spots are common on the leaves. Veins are cleared, with enations on the underside.

The potato-calico virus produces a brilliant yellow leaf mottle with dark green veinbanding in this host.

Nicotiana rustica. On this host the tuber-necrosis virus causes first a diffuse yellow mottle (Fig. 4, C). Later, concentric ring patterns and striations develop. The calico virus induces a yellow mottle with green veinbanding.

Pea. The only effect of the tuber-necrosis virus on pea is the development of black local lesions on the rubbed leaves (Fig. 4, J). The potato-calico virus causes a systemic interveinal mottle with pin-point necrotic spots in the upper leaves and a black streaking of the veins.

Cucumber. The tuber-necrosis and potato-calico viruses each cause a severe systemic yellow mottle and blotching (Fig. 4, G). The leaves are puckered and distorted. No differences between the effects of the two viruses could be detected in this host.

Celery. As illustrated in figure 4, E, celery infected with the tuber-necrosis virus develops a yellow mottle. Often yellow rings with green centers are in evidence. Later entire leaves become completely chlorotic. These symptoms are similar to those caused by the alfalfa-mosaic virus as reported by Snyder and Rich (13). The potato-calico virus induces apparently identical symptoms.

Cowpea, var. *Black-eye.* Necrotic local lesions characterize the effect of both the tuber-necrosis and potato-calico viruses on this host. As was the case with bean, the local lesions are of two distinct types. The lesions caused by the tuber-necrosis virus are small, brownish black, and solid. The calico virus lesions are large, irregular, and brick-red, and are accompanied by a red streaking of the veins.

Alfalfa. The tuber-necrosis virus occasionally causes black lesions to develop on the inoculated leaves. The systemic symptoms consist of yellow rings, streaks, and blotches on the leaves typical of the alfalfa-mosaic disease (Fig. 4, H). Often the virus could be recovered from inoculated alfalfa that had no visible symptoms.

The potato-calico virus likewise causes alfalfa-mosaic symptoms on inoculated alfalfa similar to those induced by the tuber-necrosis virus. From all inoculated alfalfa plants that had mosaic symptoms, the calico or tuber-necrosis virus could be recovered and each produced its typical symptoms in potato and bean.

PROPERTIES OF THE TUBER-NECROSIS AND POTATO-CALICO VIRUSES

Thermal inactivation. The thermal death point of the alfalfa-mosaic virus has been reported at 60°–65° C. by Pierce (10) and by Zaumeyer and Wade (16). Similar ranges have been demonstrated for the Ladino strain of the virus by Kreitlow and Price (7) and for the pepper strain by Berkeley (1). The inactivation point of a strain recovered from white clover by Johnson (5) was reported as 55°–58° C. McWhorter (8) describes a necrotic strain of alfalfa-mosaic virus from Oregon with an in-

activation point 10°–15° C. below that of the typical strains. The thermal death point of the potato-calico virus is given by Dykstra (3) as 40° C.

Three different tests were made to determine thermal inactivation of the tuber-necrosis virus, one with potato, one with pepper, and one with Bountiful beans as test plants (Table 2). In the third trial, using beans,

TABLE 2.—*Thermal inactivation of the tuber-necrosis and potato-calico viruses as indicated by systemic infection of potato and pepper and local lesions on bean*

Temperature, ° C. (10 min.)	Tuber-necrosis virus			Potato-calico virus	
	No. of infected potato plants	No. of infected pepper plants	No. of local lesions developing per bean leaf	No. of local lesions developing per bean leaf	
				California isolate	Idaho isolate
Check	5/5	6/6	65	70	60
40	5/5	4/5	.....	.....	.....
45	5/5	5/5	56	125	26
48	.....	5/5	.....	.....	.....
50	3/5	2/5	50	25	5
53	.....	.....	6	3	1
55	.....	0/5	0	0	0
60	0/5	0/5	0	0	0
65	0/5	0/5	0	0	0

the California and Idaho isolates of the potato-calico virus were included. The number of local lesions developing per bean leaf was an excellent measure of virus activity.

Juice of infected potato vines was heated in a water bath for 10 min. at the various temperatures, cooled rapidly, and inoculated immediately into the test plants, using carborundum abrasive. The results (Table 2) indicate the thermal inactivation point for the tuber-necrosis virus to be between 53° and 55° C. The two isolates of the potato-calico virus had the same inactivation point as the tuber-necrosis virus.

Longevity in vitro. Trials employing beans as test hosts showed that the two viruses are very similar in their resistance to aging. Juice was stored at 16° C. and inoculated at daily intervals. The activity of both viruses was materially reduced after 72 hr., and after 96 hr. neither was active. The tuber-necrosis virus, for example, produced an average of 75 local lesions per leaf at the outset, 50 lesions after 24 hr., five after 48 hr., two after 72 hr., and none thereafter. A longevity of 3–4 days is in good agreement with similar studies on the alfalfa-mosaic virus (13, 15).

INFECTION WITH POTATO-CALICO VIRUS PROTECTS AGAINST TUBER-NECROSIS VIRUS

To further study the interrelationship of the tuber-necrosis and potato-calico viruses, cross protection tests were performed. Potato was used as the test host since the symptoms of the two viruses differed clearly on it. Plants were inoculated first with the pure Idaho strain of the calico virus.

After 3 weeks, when the typical yellow mottle of the calico disease was visible in the top growth, the plants were inoculated with the tuber-necrosis virus. Leaves having severe calico symptoms were rubbed with juice containing the tuber-necrosis virus. At the same time comparable leaves of healthy plants of the same age were inoculated.

Twenty-seven potato vines infected with the potato-calico disease were thus inoculated with the tuber-necrosis virus. Twenty-one showed no evidence of the tuber-necrosis disease and six showed only local effects on the rubbed leaves. No calico-infected plant developed systemic infection with the tuber-necrosis virus. In the check series 25 out of 28 previously healthy plants became systemically infected with the tuber-necrosis disease. Most of these 25 plants were killed by the virus. The other three checks showed local lesions. This evidence indicates that infection with the calico virus tends to protect against subsequent infection with the tuber-necrosis virus.

Nevertheless, the two viruses often are found together in the same plant in nature. To determine whether a simultaneous inoculation of both viruses could account for this, a mixture of equal amounts of juices from tuber-necrosis and calico plants was inoculated into young potato plants. In every instance the tuber-necrosis disease appeared within the first 10 days, followed in 2 weeks by potato-calico symptoms in the young growth. Hence it is possible that, in nature, aphids feeding on a potato vine or other host infected with both strains may pick up and transmit both simultaneously.

RECOVERY OF THE TWO VIRUSES FROM NATURALLY INFECTED ALFALFA

Porter (11, 12) noted the occurrence of the potato-calico disease adjacent to alfalfa plantings, but did not confirm experimentally that alfalfa was a host. Black and Price (2), by cross protection tests, demonstrated the potato-calico virus to be a strain of the alfalfa-mosaic virus. No evidence of field spread from alfalfa to potato was presented by them.

It has been the experience of many workers that alfalfa is a poor test plant for the alfalfa-mosaic virus. A strain of alfalfa mosaic described by Kreitlow and Price (7) and one of the two strains described by Johnson (5) could not be transmitted to alfalfa. With Johnson's other strain, alfalfa could be infected only when aphid vectors were used. Further complication lies in the fact that the symptoms of alfalfa-mosaic virus in alfalfa are variable and masked under certain climatic conditions, particularly at high temperatures (14).

As previously stated, both the tuber-necrosis and calico viruses were artificially inoculated into alfalfa and recovered successfully. Each caused symptoms on alfalfa which were similar, and both were like typical alfalfa mosaic. Zaumeyer (15) found that his three different strains of alfalfa-mosaic virus all reacted identically in alfalfa.

In these investigations both potato calico and the tuber-necrosis disease

have been found important only in areas of fields adjacent to alfalfa plantings. In one particular field bordered by alfalfa on one side, it was estimated that the incidence of the calico disease was about 10 per cent in the first 200 ft. adjacent to the alfalfa, 5 per cent in the next 100 ft., and in the rest of the field just a trace. The distribution pattern of the tuber-necrosis disease in the field was identical, many plants carrying both viruses.

Circumstantial evidence certainly points to alfalfa as a carrier of both potato viruses. Attempts to establish this met with success. Inoculations of beans and potatoes with juice from alfalfa plants with typical mosaic symptoms—yellow rings and blotches—in most instances were positive. In all cases where local lesions developed on bean, definite symptoms occurred on potato. Inoculations from some mosaic-infected alfalfa plants induced typical calico in potato, from others the tuber-necrosis disease, and from still others both diseases. Juice from one alfalfa plant caused a severe necrotic disease in all ten potato vines inoculated, and the majority of the tubers produced were necrotic (Fig. 2, D and E).

Naturally infected alfalfa plants with predominant yellow rings and streaks on the leaves were more apt to contain the tuber-necrosis virus or a mixture of the two viruses. Those plants with yellow blotches more often contained only the calico virus.

A conjecture as to the cycle of development of both the tuber-necrosis and calico disease in potato fields along the margins of alfalfa fields might be as follows: 1. Old alfalfa plantings may harbor high percentages of different strains of the alfalfa-mosaic virus with no sign of damage to the crop and little evidence of symptoms. 2. When the alfalfa is cut, the pea aphid, a common alfalfa insect and a proved carrier of the virus, migrates in search of green food into the adjacent potato fields. 3. The pea aphid probably does not migrate far. It has been the author's experience that this aphid feeds poorly on potato and that attempts to induce it to reproduce on potato have failed. The vectors either perish or return to alfalfa when new growth is sufficient. 4. The localization of the calico and tuber-necrosis diseases along the margins is evidence that the vectors do not venture far from the alfalfa. 5. The occurrence of both virus strains in the same potato vine is probably the result of inoculation by an aphid or aphids that obtained both from an infected alfalfa plant.

RECOVERY FROM LADINO CLOVER OF A VIRUS CAUSING POTATO NECROSIS

It has been observed that the tuber-necrosis and calico diseases occur in the margins of potato fields bordering Ladino clover. In Ladino fields plants were noted that had bright yellow mottling and streaking of the leaves (Fig. 4, I). From such plants a virus was recovered, similar to the tuber-necrosis virus described above, that caused local and systemic necrosis in potato vines and tubers. On other hosts the reactions were in general similar to those of the tuber-necrosis virus. There were differences

that might indicate that it was a slightly different strain. For example, on pepper the symptoms of the Ladino strain were mainly puckering and distortion rather than chlorosis, and in celery there was severe stunting as well as mottle. This virus may be the same as the Ladino strain of alfalfa mosaic recently reported by Kreitlow and Price (7).

DISCUSSION

Such potato viruses as those of yellow dwarf, Canada streak, and tuber blotch, all known to cause tuber necrosis, were early in the investigations considered as possible causes of the tuber-necrosis disease. Since the tuber-necrosis virus is transmissible mechanically and by aphid, it is obviously not the yellow-dwarf virus. The tuber and vine symptoms are somewhat similar to those caused by the Canada-streak virus, a strain of aucuba-mosaic virus, as described by Dykstra (3), but in that disease tuber necrosis starts in the pith and usually is not present until 2 months after harvest. The tuber-blotch virus is also considered by Dykstra as a strain of aucuba-mosaic virus closely related to Canada streak. Dykstra was unable to obtain cross protection between either of these viruses and the potato-calico virus.

From the investigations on comparative symptomatology there would appear to be a close relationship between the tuber-necrosis and potato-calico viruses. Symptoms on various hosts further indicate that both viruses are strains of the alfalfa-mosaic virus. The local lesion reaction on bean and cowpea, the mottling and necrosis in pea, and the chlorosis on pepper are all symptoms characteristic of the alfalfa-mosaic virus. Both the tuber-necrosis and calico viruses when inoculated into alfalfa caused alfalfa-mosaiclike symptoms to develop. Finally, recovery of both viruses from naturally infected alfalfa showing the typical mosaic disease is strong evidence that both are strains of the alfalfa-mosaic virus.

Additional evidence of close relationship is the fact that the tuber-necrosis virus will not infect potato plants already infected with the calico strain. Cross protection tests by Black and Price (2) and Berkeley (1) have established that potato-calico virus is a strain of the alfalfa-mosaic virus.

The thermal inactivation of the tuber-necrosis virus is 53°–55° C. This is lower than that for most recorded strains of the alfalfa-mosaic virus. Two isolates of the calico strain were also inactivated at 53°–55° C. This is also below that of the type strain, but 15° C. above the 40° C. inactivation point for the calico virus reported by Dykstra (3).

Based upon the above evidence, the tuber-necrosis virus is considered a strain of the alfalfa-mosaic virus that is characterized principally by its ability to cause severe vine and tuber necrosis in potato. Its very close relationship to the calico strain in field occurrence, in properties, and in symptomatology suggests that it might be a mutant of the calico virus strain. Of the previously recorded strains of alfalfa-mosaic virus, prob-

ably the tuber-necrosis virus resembles most the alfalfa virus N described by McWhorter (8) from Oregon, which is characterized by its necrotic action and a thermal death point 10°–15° C. lower than that of most strains.

Only in potato fields adjacent to alfalfa or Ladino clover has the tuber-necrosis disease been found causing serious damage. It is a deadly virus on potato; once it attacks this host it nearly always kills the vines and renders most tubers unmarketable. Fortunately many infected tubers never germinate; those that do usually yield weak, nonproductive plants, many of which die early. Hence, from the standpoint of tuber perpetuation, the tuber-necrosis virus is virtually self-eliminating; and this, together with its limited spread from infected alfalfa, may minimize the importance of the tuber-necrosis virus.

SUMMARY

A disease first observed in 1946 near Stockton, California, causing severe veinal, leaf, shoot, and tuber necrosis in the White Rose potato variety, is described. Current-season infection is characterized by rugosity and necrosis of the upper leaves. Veins, petioles, and main shoots become internally necrotic. As the disease progresses, necrosis extends throughout the plant and into the tubers. Growing tips are blighted and plants are usually killed prematurely. The disease in the tuber starts as necrosis just under the skin near the stolen end and subsequently extends throughout the tuber. Necrosis is corky, dry, and dark brown. Tuber surface over affected areas becomes sunken and cracked.

Second-generation symptoms are similar but usually more severe. When necrosis involves all the eyes, infected tubers either fail to sprout or produce weak, necrotic plants.

The Mohawk, Netted Gem, Pontiac, Chippewa, Teton, and Houma potato varieties all proved susceptible upon artificial inoculation, with symptoms similar to those on White Rose. The first six of these varieties had necrotic tubers. U. S. D. A. X-immune seedling 41956, which is very susceptible, was employed as a disease source for all studies. True potato seedlings 3 weeks old were badly blighted and killed by the disease.

The disease is caused by a virus herein named the tuber-necrosis virus. The virus is tuber-perpetuated and easily transmissible by mechanical juice inoculation. Transmission by grafting and by the aphid *Myzus persicae* was also successful.

The tuber-necrosis disease usually occurs in the margins of potato fields adjacent to alfalfa plantings and frequently is associated with the calico disease of potato. Often vines showing symptoms of both diseases are encountered. From such plants both viruses could be recovered and were separated by repeated subinoculations. The close relationship of the calico and tuber-necrosis viruses in nature prompted comparative studies of the two.

Symptoms of the potato-calico virus on potato are principally chlorotic

blotching of the leaves with no tuber necrosis, whereas the tuber-necrosis virus causes shoot and tuber necrosis with no yellow blotching.

Both viruses when inoculated into alfalfa cause alfalfa-mosaiclike symptoms to develop.

Symptoms on pepper, *Datura stramonium*, cucumber, and celery caused by the two viruses were identical—in all cases being a systemic leaf mottle.

Neither the calico nor tuber-necrosis virus infected tomato.

On three species of tobacco, the tuber-necrosis virus causes ring, striation, and leaf patterns, whereas the potato-calico virus induces a bright yellow mosaic with green veinbanding.

Local lesions are the principal effect of both viruses on bean and cowpea.

Symptomatology studies show a close relationship between the tuber-necrosis and calico viruses and indicate that both are strains of the alfalfa-mosaic virus.

The thermal inactivation of the tuber-necrosis virus is 55° C.; its longevity *in vitro* at 16° C. is 3–4 days. Properties of the calico virus were identical.

Inoculation of potato with potato calico protects against the tuber-necrosis virus. Simultaneous inoculation of potato with the two viruses using juice containing an approximately equal amount of each virus resulted in infection with both viruses as indicated by symptoms.

Both the calico and tuber-necrosis viruses were recovered from alfalfa plants naturally infected with alfalfa mosaic. Inoculations of potato showed that mosaic-infected alfalfa plants may harbor either or both potato viruses.

A virus similar to the tuber-necrosis virus was recovered from yellow-blotched Ladino clover. Inoculations of potato, bean, and other hosts with the Ladino-clover virus produced a reaction similar but not identical to that of the tuber-necrosis virus.

The tuber-necrosis virus is considered to be a strain of the alfalfa-mosaic virus that is characterized by its ability to cause vine and tuber necrosis in potato.

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MILD RUGOSE MOSAIC OF SWEET CHERRY[1]

G. H. Berkeley

(Accepted for publication March 29, 1950)

In August, 1942, foliage of two Black Tartarian cherry trees in a lawn border row near Grimsby, Ontario, had a distinct dull green mottle with chlorotic spots and rings. Otherwise these trees appeared to be normal, though the grower stated that they did not bear satisfactory crops.

As the symptoms were suggestive of a virus disease, bud sticks were secured and five peach seedlings were budded with this material. In 1943 the inoculated trees had definite chlorotic spot mottling of the foliage, with a few necrotic spots. No further inoculations were attempted until 1944 and again in 1946, when transfers were made from the inoculated peach trees to several different hosts. This paper describes the symptoms on these hosts.

The disease in question, for which the name mild rugose mosaic is proposed, is of no economic importance at the present time, and no cases have been brought to our attention since the original one in 1942. However, the unproductiveness of the parent trees and the rather severe symptoms resulting from inoculations indicate that the disease is a potential cause of serious loss in sweet cherries.

DIFFERENTIAL HOSTS

The hosts used were *Prunus persica* L., varieties Elberta and Rochester, and peach seedlings; *P. cerasus* L., variety Montmorency; *P. avium* L., varieties Black Tartarian and Napoleon; *P. domestica* L., varieties Italian prune and Lombard plum; and *P. mahaleb* L.

Since virus-free trees are a prerequisite with investigations of virus diseases, trees of the above varieties were established at the St. Catharines Laboratory from specially selected root stocks and indexed scion material. The seedling root stocks were grown for 1 year previous to budding and showed no symptoms of disease. The scion material of the above varieties had been indexed previously on peach and gave no reaction, thus indicating freedom from viruses. It was assumed, therefore, that these trees were free from recognizable viruses.

INOCULATIONS

Inoculations were effected by budding these special trees in August of the respective years.

In this paper, no distinction is made between the 1944 and 1946 inoculations; no appreciable differences in symptoms were noted between

[1] Contribution No. 1005 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

the two series. Two to five trees of each variety were inoculated in each series.

SYMPTOMS ON HOSTS

Sweet cherry: Black Tartarian and Napoleon. In the spring following

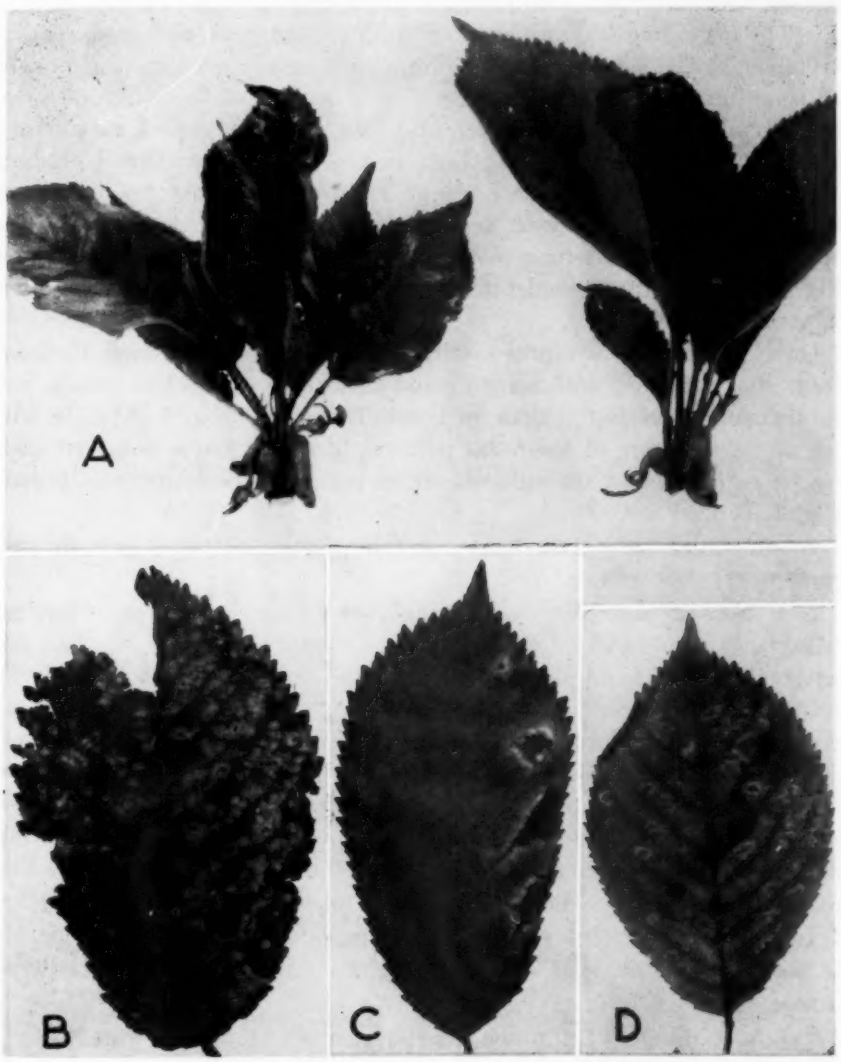

FIG. 1. Symptoms of mild rugose mosaic on sweet cherries. A. Left, stunted, misshapen, and chlorotic unfolding leaves from diseased bud; right, foliage from healthy bud. B. Chlorotic spotting and rings on Black Tartarian variety. C. Chlorotic patterns and rings on Napoleon variety. D. Late-season chlorosis on Black Tartarian.

budding, the first symptom on Black Tartarian was a delay in foliation, largely confined to the branches with the inserted diseased buds. Unfolding leaves had a mild waviness of margins and a definite chlorotic spot mottle with rings and partial rings. Leaves formed later appeared to be

normal or showed very mild chlorotic spot symptoms. As the season advanced the symptoms tended to fade, but did not entirely disappear.

During the second and third years, the symptoms were more pronounced. The delay in foliation spread to several other branches and the buds on these branches had a ragged appearance as they opened (Fig. 1, A). The unfolding leaves had wavy margins and were puckered, stunted, misshapen, and distorted, with chlorotic spots, rings, partial rings, bands, and some necrotic tissue, especially in the centers of the chlorotic areas. On individual leaves the number and size of spots varied considerably (Fig. 1, B). Leaves unfolding later in the season also showed chlorotic spots, rings, and bands, but little or no distortion. As the season advanced and more new growth developed, the trees took on a more normal appearance, but the chlorotic mottling remained in a less pronounced form (Fig. 1, D). Only a moderate percentage of the leaves showed these symptoms.

On Napoleon, the symptoms were similar to those on Black Tartarian except that the wavy leaf margins and distortion in the first season were slightly more pronounced than on Black Tartarian (Fig. 1, A). In addition, slight tattering of the basal portions of a few leaves occurred and a greater percentage of the chlorotic rings tended to be larger and broader (Fig. 1, C, and 2, C).

As the inoculated trees have not fruited, the effect, if any, on fruit production is not known.

Montmorency sour cherry. In the year following budding, there was a slight delay in foliation and a small percentage of the leaves had mild mottle with vein banding, rings, and chlorotic spots (Fig. 2, A). The leaves from a few opening buds had wavy margins and puckered surfaces. In the second and third years, the delay in foliation was more pronounced. Also the unfolding leaves of a greater percentage of buds were stunted, puckered, distorted, and twisted. The markings on the leaves were more general and more pronounced, consisting of chlorotic rings, bands, blotches, vein banding, and coarse spots (Fig. 2, B). In the third year, a few leaves also had mild necrotic etching on the upper surfaces.

In each of the three years, the leaf markings became less evident as the seasons advanced, but did not disappear entirely. Only a small percentage of the leaves had symptoms.

Peach seedlings. On peach seedlings, a slight to moderate delay in foliation occurred in the year following budding. As the leaves unfolded, a definite chlorotic spot mottle appeared and a small percentage of the spots became necrotic. The leaves generally were reduced in size and the plants as a whole were mildly stunted. In the second and third years, the chlorotic mottling recurred and the internodes on a few branches became moderately shortened. When the peach seedlings were cut back above the inserted diseased bud, the new shoots were moderately stunted, with a

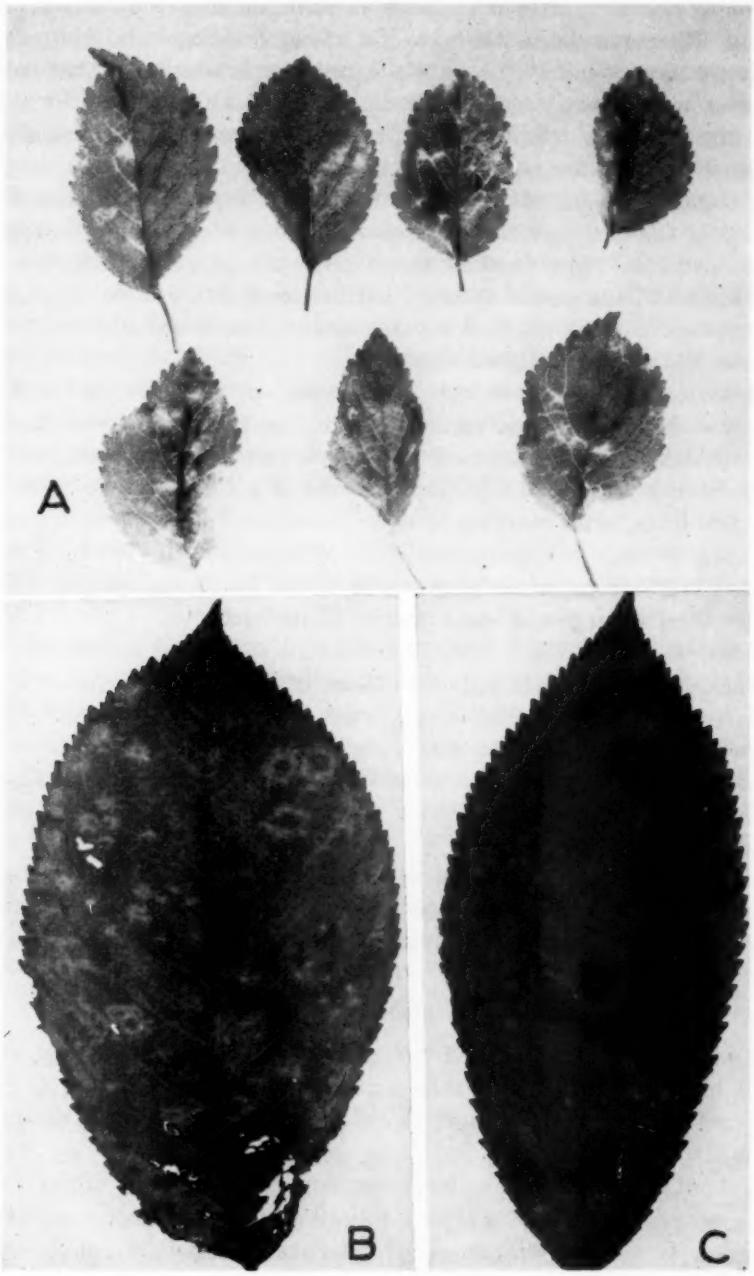

FIG. 2. Symptoms of mild rugose mosaic on sweet and sour cherries. A. Unfolding leaves from buds of Montmorency sour cherry. B. Chlorotic rings, spots, and bands on Montmorency sour cherry. C. Chlorotic spotting on Napoleon sweet cherry.

moderate shortening of internodes. The foliage showed the same chlorotic markings as those on the seedlings that were not cut back.

Elberta peach. Little or no delay in foliation occurred on one- or two-year-old Elberta trees in the year following budding, and the chlorotic mottling was very mild. There was a mild general stunt of the tree and the leaves were slightly smaller than those on healthy trees. By the fall of the first season, a definite roughening and splitting of the bark occurred on a small area of the trunk.

In the second and third years, the chlorotic mottling was more pronounced in the early part of the season. Some of the chlorotic spots became necrotic and the necrotic tissue fell out. By this time, the leaves were about half the size of those of normal trees of the same age. Roughening and splitting of the bark became more extensive, and internodes on a few branches were slightly shortened.

Rochester peach. Much the same symptom sequence as on Elberta peach was obtained on this variety, except that in the first year there was moderate delay in foliation and this became more pronounced in the second and third years. In addition, the tips of a few branches were killed, and a few buds, after starting to open, wilted and died.

Italian prune. No symptoms were obtained on this host. Transfers were not made from prune to peach or Black Tartarian trees to ascertain whether the Italian prune was a carrier of the virus.

Lombard plum. No definite or consistent symptoms were apparent on this variety. In one tree only was there a suggestion of mild shortening of internodes on two branches. A transfer was made from one of these inoculated Lombard trees to peach seedlings, and the peach reacted with a general stunting of the leaves and a definite chlorotic mottling of the foliage. This result might indicate that Lombard plum may act as a carrier of this virus.

Prunus mahaleb. Seedlings of this species were not inoculated, but *P. mahaleb* shoots from the rootstock of inoculated and symptom-expressing Napoleon trees had chlorotic mottling, line patterns, and "oak leaf" symptoms.

DISCUSSION

The symptoms as described for mild rugose mosaic on sweet cherries do not indicate close relationship with any other virus disease so far encountered in Ontario. It must, therefore, be considered as a disease new to this Province.

It should be pointed out, however, that the symptoms of mild rugose mosaic on peach, namely delay in foliation, dieback (Rochester variety), chlorotic spot mottle, shortening of internodes, and bark roughening are similar to, though much milder than, those associated with necrotic ring spot and cherry yellows on this host.[2] Also on sweet cherry a ring spotting is common to these three diseases.

[2] Berkeley, G. H., and R. S. Willison. Yellows and necrotic ring spot of sour cherries in Ontario. Phytopath. 38: 509–518. 1948.

This similarity in type of symptoms, especially on peach, might indicate a possible relationship between mild rugose mosaic and necrotic ring spot and cherry yellows; but when the symptoms of these diseases are compared with those of mild rugose mosaic on other hosts, the possibility of a close relationship becomes remote. For instance, the rugosity associated with mild rugose mosaic on sweet cherry is lacking with necrotic ring spot and cherry yellows on this host. In addition, when Italian prune and Lombard plum were inoculated with the mild rugose mosaic virus, the lack of necrotic spotting in the first year and the absence of prune dwarf-like symptoms subsequently would indicate the absence of the necrotic ring-spot virus and the cherry-yellows virus, or virus complex, respectively. Moreover, the lack of acute necrosis on unfolding leaves and the absence of yellowed and cast leaves on Montmorency sour cherry inoculated with the mild rugose mosaic virus would again indicate the absence of the necrotic ring-spot and cherry-yellows viruses, respectively. Also the recurrence of mottle on peach would indicate the presence of a virus other than the necrotic ring-spot virus.

These results would also suggest that the ring spotting associated with mild rugose mosaic on sweet cherry was not caused by the necrotic ring-spot virus.

It is concluded, therefore, that the necrotic ring-spot and cherry-yellows viruses are not associated with the mild rugose mosaic virus, in spite of the similarity of certain symptoms on peach and the presence of ring spotting on sweet cherries.

Though the symptoms of peach ring-spot[3] and mild rugose mosaic on peach have certain characteristics in common, the recurrence of symptoms on peach with mild rugose mosaic would indicate that these two diseases are dissimilar.

Mottle leaf, as described for sweet cherries on the West Coast, resembles mild rugose mosaic, especially in the early part of the season when trees are leafing out, the ragged buds and the chlorotic and distorted foliage being common to both diseases. However, these symptoms, especially the chlorotic mottling and distortion of foliage, are much milder with mild rugose mosaic, and the delay in foliation associated with mild rugose mosaic is lacking in mottle leaf.

Moreover, a comparison of the symptoms produced by the mild rugose mosaic virus with those described by Reeves[4] for the mottle-leaf virus on Black Tartarian sweet cherry, Montmorency sour cherry, and peach, would indicate that these two diseases are dissimilar. The mottle-leaf virus causes severe symptoms on Napoleon, but extremely mild to fugitive symptoms on Black Tartarian; whereas inoculations with the mild rugose mosaic virus produced prominent symptoms of about the same order on both of

[3] Cochran, L. C., and L. M. Hutchins. A severe ring-spot virosis of peach. (Abstr.) Phytopath. 31: 860. 1941.

[4] Reeves, E. L. Mottle leaf, a virus disease of cherries. Jour. Agr. Res. [U.S.] 62: 555–572. 1941.

these varieties. Reeves obtained on Montmorency only an occasional chlorotic spot on a few leaves, with absence of leaf ruffling; whereas with mild rugose mosaic, there was delay in foliation, distortion of unfolding leaves, and conspicuous chlorotic spotting. Moreover, on peach, the mottle-leaf virus caused only mild chlorotic spotting on a few leaves, whereas the mild rugose mosaic virus caused delay in foliation, prominent chlorotic spotting on many leaves, mild shortening of internodes, and bark roughening.

The rugosity of unfolding leaves as described by Thomas and Rawlins[5] for cherry mosaic 1 might indicate a relationship to mild rugose mosaic, but their description of symptoms is too meager to permit of definite comparisons. However, their illustration of affected leaves, and the casting of chlorotic areas from leaves, and later the affected leaves themselves, would indicate that these diseases are dissimilar.

Whether or not mild rugose mosaic is caused by a single virus or by a virus complex is not known. If a complex, one might suspect one entity to be a strain of the ring-spot group of viruses because of their widespread existence in stone fruits and their known association with certain other virus diseases on stone fruits. Nevertheless, the evidence submitted in this paper would indicate the absence of any known strain of the necrotic ring-spot virus.

SUMMARY

A mild-rugose-type mosaic virosis, found originally on two Black Tartarian sweet cherry trees, has been transferred by budding to varieties of peach, plum, and cherry.

On Black Tartarian and Napoleon sweet cherries, the outstanding symptoms were distortion, stunting, and chlorotic mottling of the unfolding leaves each year. Chlorosis occurred as spots, rings, bands, and patterns. A low percentage of later-formed leaves showed chlorosis of a milder type. There was considerable variation in symptoms on individual leaves.

On peaches, there was delayed foliation and spot mottling of foliage with mild bark roughening and splitting, especially on Elberta and Rochester varieties.

Symptoms on Montmorency sour cherry comprised delay in foliation, distortion of unfolding leaves, and chlorotic spots, rings, and bands. Inoculations on Italian prune and Lombard plum gave no discernible symptoms.

Mild rugose mosaic is considered to be an undescribed disease since its symptoms on the above hosts do not agree in all respects with those of any known virus disease.

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[5] Thomas, H. E., and T. E. Rawlins. Some mosaic diseases of prunus species. Hilgardia 12: 623–644. 1939.

THE ELECTRON MICROGRAPHY OF THE WISCONSIN PEA-STREAK VIRUS[1]

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INTRODUCTION

The prevalence of Wisconsin pea streak was first observed by Walker and Hare (7) in 1942. Detailed studies of the disease were recently reported by Hagedorn (1) and Hagedorn and Walker (2, 3). The most conspicuous symptom incited by the pea-streak virus was a brown necrotic streaking of the stem and petioles. The physical properties of the virus in crude extracts, especially high tolerance to dilution (1 to 1,000,000) and resistance to aging *in vitro* (16 days) suggested that it might readily lend itself to purification and to study with the electron microscope. This paper is to report the preliminary purification of the virus by differential centrifugation and to present electron micrographs which revealed its unusual, elongated, threadlike form.

METHODS AND MATERIALS

Source of Diseased Tissue

The Perfected Wales pea, a very susceptible variety, was used for the source of diseased tissue. A 260-ft. row of these peas was planted May 14 near Madison. When they had reached a height of 12 in., the plants on the southern 150 ft. of the row were inoculated with the Wisconsin pea-streak virus. This was the same virus previously studied by Hagedorn and Walker (2, 3) and had been maintained on pea plants in the greenhouse. Inoculation in the field was accomplished by rubbing, with an infective cheesecloth pad, two leaves of each plant which had been previously dusted with carborundum. The plants on the northern 110 ft. of the row were left uninoculated.

Twenty days after inoculation, symptoms of Wisconsin pea streak were apparent on the inoculated plants. By this time they had grown to about 22 in. in height, and some were almost dead from severe disease development. Diseased plants chosen for the isolation of the virus were still in a very succulent state but showed severe symptoms, i.e., stem streak, node browning, and some apical foliage malformation. About a bushel of the above ground portion of these diseased plants was harvested and a like

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amount of plants without visible symptoms was harvested from the uninoculated portion of the row.

Fourteen days after harvesting, it was noted that most of the plants remaining in the northern, uninoculated portion of the row had typical symptoms of Wisconsin pea streak. Apparently a vector had spread the virus along the row. Thus, the question arose as to whether there might have been virus present in the apparently healthy plants removed for the initial study. Consequently it was decided to make a study of other healthy pea tissue. The material used for this work was obtained from Wisconsin Perfection peas growing in another experimental plot at Madison. No virus disease symptoms were observed on peas growing in this location. The healthy peas taken from this plot were of about the same age and maturity as those used earlier.

Preparation

Immediately after harvesting, the plants were placed in a room maintained at –8° C. The following morning, the frozen plant tissue was macerated in a meat grinder. The juice was expressed from the resulting pulp through several thicknesses of cheesecloth. It was clarified by low-speed centrifugation ($2800 \times G$ for 15 min.). Although the insoluble tissue fragments still retained most of the virus, as was shown by resuspending the pulp and inoculating back to peas, only the clarified juice was used for the preparation of the virus for the electron photomicrographs.

Two-hundred-ml. portions of the clarified juice were then centrifuged in the batch-type rotor of a Sharples centrifuge at $50{,}000 \times G$ for 45 min. The sediment was collected on a sheet of filter paper, under which was placed a sheet of cellulose acetate to aid in removing the wet filter paper from the rotor. The virus was thrown down in the blue-gray sediment. This was scraped from the filter paper with a rubber spatula aided by a fine stream of water and suspended in 35 ml. of water.

The insoluble particles were removed from this suspension by low-speed centrifugation. The resulting liquids from infected plants were opalescent in appearance; those from healthy plants were darker in color and lacked opalescence. The final centrifugation was carried out in a Grebmeier air-driven spinning-top centrifuge. This was done in six 2-ml. cellulose nitrate tubes with the centrifuge operating at about $100{,}000 \times G$ for 45 min. The resulting pellets were combined, suspended in 1.9 ml. of water, and again centrifuged at low and then at high speed. The final pellet from the infected tissue was white and translucent. That from the healthy pea tissue was much smaller in volume and rather opaque.

The final pellets were suspended in 2 ml. of water and aliquots were taken for the preparation of mounts for the electron micrographs and for assay for virus infectivity. For the electron micrographs, an aliquot was diluted with from 400 to 2,000 parts of water and a drop placed upon the electron microscope screens which were covered with a collodion film. The

screens were previously cooled to −8° C. so that the drop froze soon after being placed on the screen. The mounts were dried while still frozen by allowing them to stand overnight in a desiccator over calcium chloride at −8° C. The dried mounts were then shadow-cast with uranium and viewed in an R. C. A. electron microscope. About 100 electron micrographs of the pea streak virus were taken. Some typical micrographs of the mounts prepared from infected and healthy pea plants are reproduced herewith.

The assay of the final pellets for virus infectivity was carried out by diluting ½ ml. of the suspension of the pellet with 2 ml. of water and inoculating to greenhouse-grown pea plants. At the same time, a control series was inoculated with sap from freshly macerated infected plants with severe symptoms.

EXPERIMENTAL RESULTS

The electron micrographs prepared from the pellets obtained from plants with severe symptoms of the Wisconsin pea streak disease showed an abundance of long threadlike particles. Mounts made in the same manner from healthy pea plants showed no such particles. Figure 1, A and B, and figure 2, A, are typical of those micrographs obtained from plants with severe symptoms of the disease. Figure 2, B, shows particles from plants from the northern, uninoculated part of the row which later proved to be infected. It would appear that most of the particles in this micrograph are somewhat shorter than the majority of those in figure 2, A. Figure 3, A, is an electron micrograph typical for healthy pea plants. Such mounts from healthy tissue showed a small amount of normal spherical particles but no elongated threadlike particles that were so abundant in mounts from infected tissue. Figure 3, B, shows a mount of the same virus preparation used for figure 1 and figure 2, A, after storage of an aqueous solution for 3 months at 4° C.

Measurements on several electron micrographs showed that the particles had very uniform width, but great variation in length. The length was often several hundred times greater than the width. Measurement of several threadlike particles indicated a width between 10 and 14 $m\mu$. This is slightly narrower than the virus particle of tobacco mosaic virus (5). Particles as short as 150 $m\mu$ were occasionally seen; on the other hand, what appeared to be single threadlike particles longer than 5000 $m\mu$ were often observed. A plot of the lengths of all the particles from several electron micrographs failed to show any predominance of a common length; rather the lengths of the particles seemed to follow a random distribution over a wide range.

When ⅓ of the final pellet obtained from diseased plants was suspended in 2 ml. of water and inoculated to healthy pea plants, 88 per cent of the plants developed typical symptoms of the Wisconsin pea-streak disease. At the same time, the control series in which inoculation was made with freshly expressed sap from diseased plants gave 63 per cent infection.

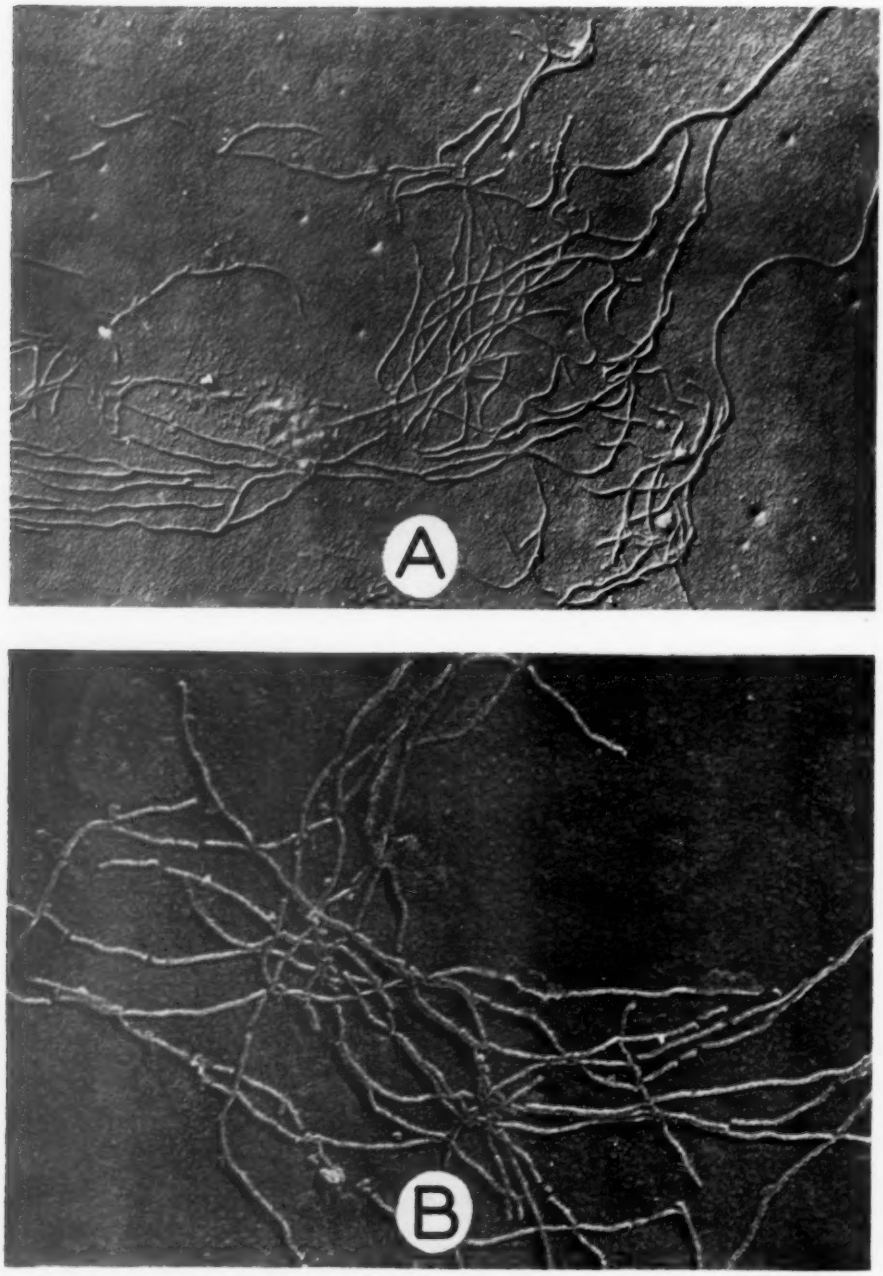

FIG. 1. Electron micrographs of the typical long, threadlike particles from plants with severe symptoms of the Wisconsin pea streak disease. A, 21,400×. B, 49,500×.

DISCUSSION AND SUMMARY

Many electron-microscopic studies of virus-infected plants have revealed the presence of characteristic particles associated with a particular virus

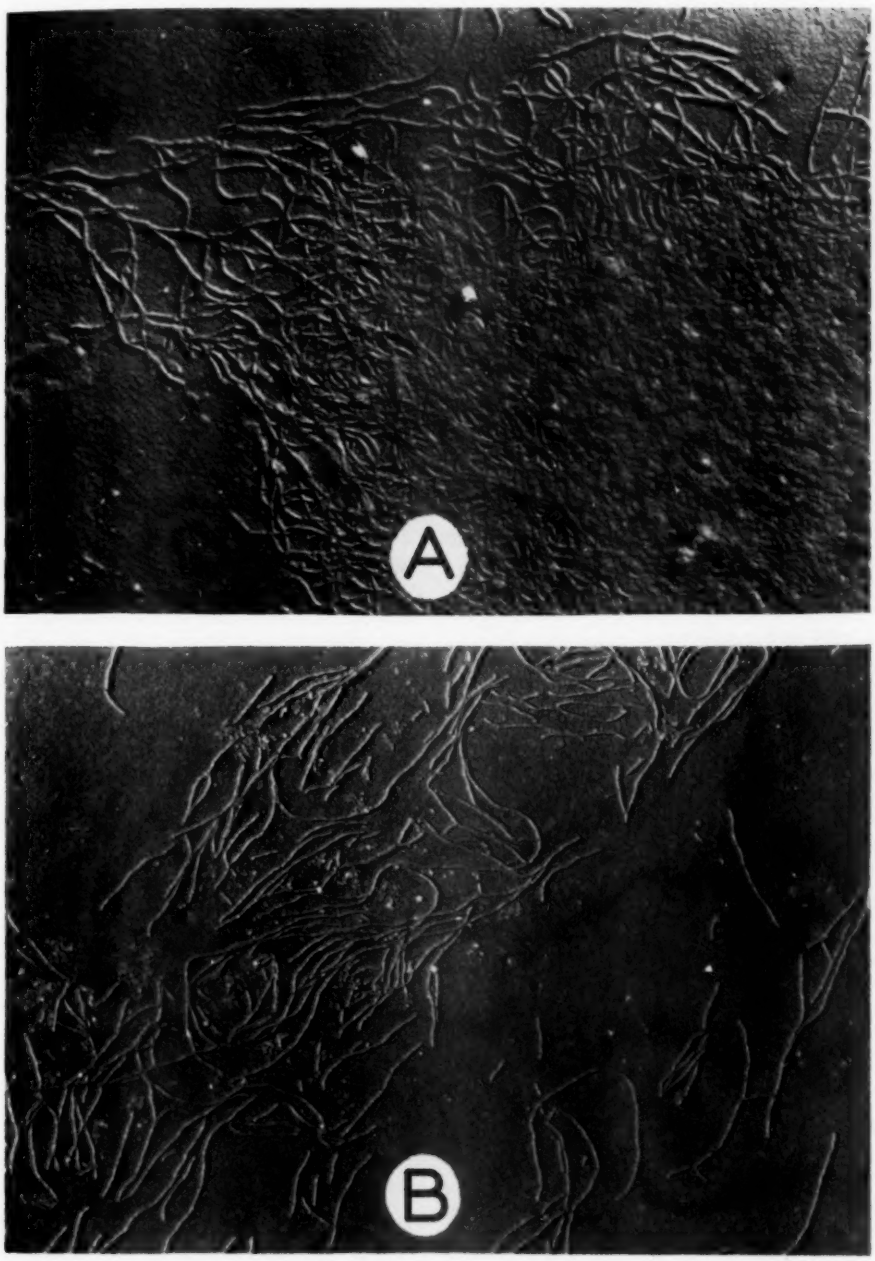

FIG. 2. A. Electron micrograph of a network of the Wisconsin pea streak virus particles. (24,800 ×.) B. Micrograph of the particles from plants just prior to symptom expression. Note that the particles in this case are somewhat shorter and show less tendency toward aggregation. (22,800 ×.)

disease. Although the plant virus particles thus far described vary in size and shape, they are in general either spherical or rod-shaped (4). Tobacco-

mosaic virus, which is typical of the latter viruses, generally has a predominance of particles about 15×280 $m\mu$ (5), although longer particles, presumably aggregates of this basic unit, are often observed. Takahashi (6) has recently published electron micrographs of the *Brassica nigra* virus which show particles about 12×700 $m\mu$.

One of the most striking aspects of the electron micrographs of the Wisconsin pea-streak virus was the unusual morphology of the virus particles: long, threadlike, filamentous, flexible particles, often several thousand $m\mu$ in length, are abundant in mounts from plants showing symptoms of the Wisconsin pea-streak disease but are absent in those from healthy pea plants. It would thus appear that these threadlike particles represent the virus of the Wisconsin pea- streak disease; their presence in infected plants just prior to symptom expression would support this view.

Lack of any predominance in common uniform length of particles makes it impossible to assign a size to the basic infective unit of these particles as has been done with many other viruses. If such a basic unit exists, it would appear that the elongated threadlike particles seen in the electron micrographs must be produced by an end-to-end aggregation. That the ends of these particles possess a chemical structure that has a high affinity or reactivity for combining with the ends or with the sides of other virus particles is apparent from the electron micrographs. If the ends of the virus particles lacked this added reactivity, the number of free ends of the elongated particles should be equal to twice the number of particles, since each particle would have two free ends. However, a count of the "individual" particles (those whose ends are free or form an angle with other particles to which the ends appear to be attached) from four electron micrographs showed that 608 "individual" virus particles had only 72 free ends instead of the 1216 which would be expected if the ends were not combining. Thus, as these counts indicate, the Wisconsin pea-streak virus readily forms a random network by an end-to-end and an end-to-side aggregation. Figure 2, A, illustrates such a network.

In the electron micrograph of extracts from naturally infected plants prior to symptom expression (Fig. 2, B), the particles at this stage of disease development appeared to be shorter than those from plants showing severe symptoms of the disease. A count of the number of "individual" particles and of ends showed that at this stage the ratio of ends to total particles was about four times greater than from plants wih severe symptoms. This suggests that the virus particles undergo an aggregation in the plant during the development of the disease.

The virus particles also appear to undergo an aggregation when solutions of the purified virus in distilled water are stored at 4° C. Figure 3, B, illustrates this type of aggregation. Its most striking feature is the extreme end-to-end aggregation which sometimes produced threadlike fibers too long to measure in the electron micrographs. It appears that under these con-

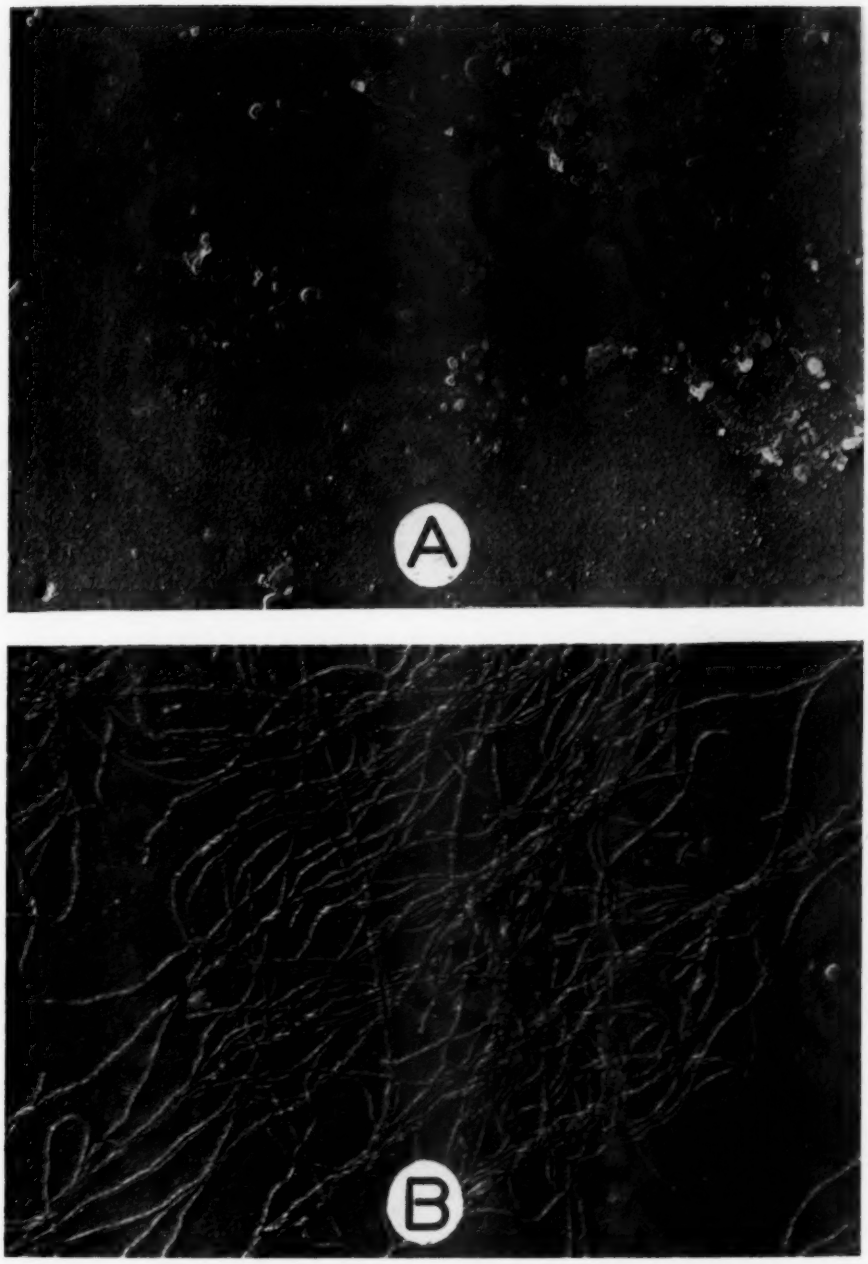

FIG. 3. A. Electron micrograph typical of those obtained from healthy pea plants. (21,700×.) B. Micrograph of Wisconsin pea streak virus particles after 3 months' storage at 4° C. (29,900×.)

ditions, side-to-side aggregation also occurs to produce bundles containing several parallel strands of the virus particles. Often these strands were observed to coil about each other as do the strands of a rope.

It should be emphasized that infectivity tests show that most of the virus of the Wisconsin pea-streak disease is associated with the insoluble tissue fragments. For such tests, healthy pea plants were inoculated with the clarified aqueous extracts and with suspensions of the insoluble tissue fragments from heavily infected plants. The tissue suspensions usually produced a high percentage of transmission, whereas the clarified aqueous solutions often showed little or no transmission. This would suggest that the virus particles must be rather firmly combined with large tissue fragments which are removed when the extracts are clarified. We have recently obtained electron micrographs which show nearly all of the threadlike particles of the virus attached to larger masses of tissue. These micrographs will be presented in a later paper.

The shape of the threadlike particles associated with the Wisconsin pea-streak disease shows that the virus nucleoproteins are capable of existing in a fibrous form as well as in the spherical and rod-shaped forms that are generally observed. The occurrence of virus particles in the fibrous form as well as the spherical and rod-shaped forms serves to emphasize the close relationship between the infective viruses and other proteins.

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INHERITANCE OF SYMPTOM EXPRESSION OF POD MOTTLE VIRUS

H. Rex Thomas and W. J. Zaumeyer

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Pod mottle was recently described as a new virus disease of beans (*Phaseolus vulgaris* L.) by Zaumeyer and Thomas (5). The virus produced either local lesions or a systemic mottle on each of the numerous bean varieties tested, an effect similar to that produced by the southern bean mosaic virus described by Zaumeyer and Harter (3).

Homozygous varieties or strains of beans that manifested local lesions of pod mottle virus (Fig. 1, B), although susceptible to the virus at points of

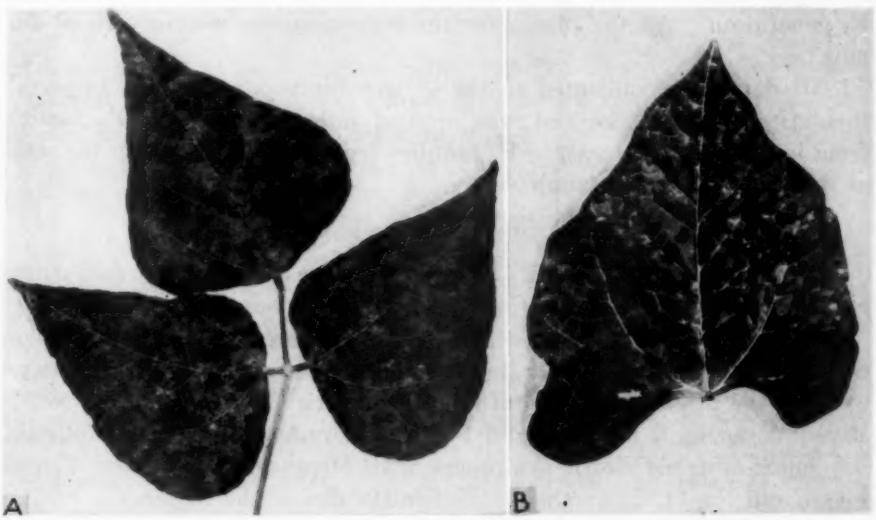

Fig. 1. Pod mottle. A. Systemic mottle on trifoliate leaves of the Longreen variety of bean. B. Local lesions produced on leaves of the Idaho Pinto variety of bean.

inoculation, were immune from systemic infection. The virus could be recovered from the inoculated leaves having local lesions but not from any other portion of the plant. All the plants that did not show the local lesion type of infection developed the systemic mottling (Fig. 1, A) about 10 days after inoculation. The virus was recovered from all portions of systemically infected plants, and juice extracted from such plants always produced necrotic lesions when inoculated to susceptible varieties on which local lesions occur. No variety was immune from the virus.

The studies reported herein deal with the inheritance of local lesion and systemic responses of the plant to virus infection as indicated by the behavior of the F_1, F_2, and F_3 hybrids inoculated with pod mottle virus.

MATERIALS AND METHODS

The virus used in these studies was isolated from infected beans growing in a greenhouse at Beltsville, Maryland.

The crosses were made under controlled greenhouse conditions. In addition to crosses made specifically to study the inheritance of the symptom expression of pod mottle virus, certain other crosses in the F_2 generation which had been made for other purposes were used. No F_1 generation plants of these latter crosses were available.

The plants were inoculated in the greenhouse. The general procedures of inoculation, etc., were similar to those reported previously (3). The records for local lesion response were made 4 or 5 days after inoculation, and for systemic response, 10 to 15 days after inoculation. In some instances the F_2 plants were grown in 4-in. pots and in others the seed was planted directly in 10-in. pots. Only a limited number of the F_2 plants were grown to maturity in the greenhouse for production of seed for the F_3 generation. All the plants for the F_3 generation were grown in 4-in. pots.

All data were subjected to the χ^2 test for goodness of fit to certain theoretical ratios. The test was applied independently for F_2 families from individual F_1 plants, to F_3 families from F_2 plants, and to the totals of a number of tested families.

REACTION OF SELFED PARENTS

When inoculated artificially in the greenhouse a variety or strain showed either local-lesion infection or systemic infection, but never both.

From 12 to 20 plants of each of the selfed parents used in the various crosses were inoculated with the pod mottle virus. Local lesions developed on the inoculated leaves of Great Northern U. I. No. 1, Pinto No. 14, Rival, U. S. No. 5 Refugee, and Pinto (Colorado strain). The following developed systemic mottle symptoms: Full Measure, Red Kidney, Tendergreen, and No. 1197 (number carried in the files of the writers).

REACTION OF F_1 GENERATION

Ten F_1 plants from crosses between local lesion and systemic type parents were tested. These consisted of two plants each from the crosses Great Northern U. I. No. 1 × Tendergreen and the reciprocal, U. S. No. 5 Refugee × Tendergreen and the reciprocal, and one plant each from Rival × Full Measure and the reciprocal. All of the F_1 plants developed the local-lesion type of symptom.

REACTION OF F_2 GENERATION

An analysis of the F_1 and F_2 data show that the local-lesion type of infection (Fig. 1, B) is dominant to the systemic-mottle type (Fig. 1, A). In the F_2 a 3:1 segregation resulted, indicating a single-factor difference (Table 1). The reciprocals behaved alike. The χ^2 values indicated that

TABLE 1.—*Reactions of F_2 progenies when inoculated with pod mottle virus*

Parentage of cross	Plants inoculated	Plants showing		χ^2 for 3:1 ratio[a]
		Local-lesion infection	Systemic mottle infection	
	Number	*Number*	*Number*	
Pinto No. 14 × Red Kidney	84	66	18	0.571
Red Kidney × Pinto No. 14	118	89	29	0.011
U. S. No. 5 Refugee × Tendergreen	97	72	25	0.031
Tendergreen × U. S. No. 5 Refugee	96	70	26	0.222
Great Northern U. I. No. 1 × Tendergreen	311	226	85	0.911
Tendergreen × Great Northern U. I. No. 1	52	39	13	0.000
Full Measure × Rival	61	47	14	0.137
Rival × Full Measure	62	50	12	1.054
Pinto (Colorado strain) × 1197[a]	81	63	18	0.333
1197[b] × Pinto (Colorado strain)	92	63	29	2.087
Total	1054	785	269	0.153

[a] 5 per cent point 3.841.
[b] Numbers carried in files of the writers.

the observed data fit the calculated rather closely for each of the ten crosses. Of a total of 1054 plants, 785 showed local-lesion necrotic infection, and 269, systemic infection. This segregation is a close fit to a 3:1 ratio, with a χ^2 value of 0.153. This is well below the 5 per cent point of 3.841.

REACTION OF F_3 GENERATION

Of 66 F_3 families derived from F_2 plants that showed the local-lesion type of infection, 18 were homozygous for the local-lesion type of infection and 48 were heterozygous when inoculated with pod mottle virus (Table 2).

TABLE 2.—*Reactions of F_3 progenies descended from previously tested F_2 progenies when inoculated with pod mottle virus*

Classification in F_3 generation	Families tested[a]	Plants	Plants showing	
			Local-lesion infection	Systemic mottle infection
	Number	*Number*	*Number*	*Number*
Homozygous local-lesion type	18	317	317	0
Heterozygous local-lesion type				
Observed	48	1046	775	271[b]
Calculated 3:1 ratio	.....	.....	784.5	261.5
Homozygous systemic-mottle type	24	460	0	460

[a] χ^2 for 1:2 distribution of families = 0.764; 5 per cent point = 3.841.
[b] χ^2 for total population was 0.460; 5 per cent point = 3.841.
Accumulated χ^2 was 23.138; 5 per cent point = 64.934 for 48 degrees of freedom.

The deviation from the calculated 1:2 ratio of homozygous to heterozygous local-lesion type families was nonsignificant, with a χ^2 of 0.764. In the 48 heterozygous families, the segregation of plants with the local-lesion type of infection and plants with the systemic-mottle type showed a good fit to a 3:1 ratio (Table 2). The progenies from 24 F_2 selfed families of the sys-

temic-mottle type of infection were homozygous for systemic infection (Table 2). These results furnish additional proof in support of the single-factor hypothesis.

DISCUSSION

The results demonstrate that in the hybrids investigated the inheritance of symptom expression of pod mottle virus is controlled by a single genetic factor difference and that the local-lesion infection is dominant to the systemic-mottle type. The reaction of the heterozygous plants was indistinguishable from that of plants homozygous for the local-lesion type of infection.

Zaumeyer and Harter (4), studying the inheritance of virus expression in beans infected with southern bean mosaic virus, and Holmes (1, 2), studying the inheritance of the localization of tobacco mosaic virus in certain pepper varieties and the inheritance of resistance to tobacco mosaic in tobacco, reported similar responses.

Immunity from pod mottle virus has not been observed in any variety tested. Forty-one varieties were found that possess the dominant character for local-lesion expression (Fig. 1, B). These varieties can be considered as commercially resistant since little or no damage to the plant occurs from this type of infection. Fortunately most of the varieties resistant to common bean mosaic virus are resistant to systemic infection by the pod mottle virus. In the development of new bean varieties resistant to common bean mosaic, attention should be given to selecting progenies resistant to systemic infection of both common bean mosaic and pod mottle virus. Resistance to southern bean mosaic could not be so easily incorporated because many of the varieties resistant to common bean mosaic possess the recessive factor for resistance to southern bean mosaic and are susceptible to the systemic type of infection.

SUMMARY

The inheritance of the expression of symptoms of pod mottle virus was found to be governed by a single allelomorphic pair of Mendelian factors. Plants carrying the dominant allelomorph are susceptible to a local-lesion type of infection. The homozygous recessive plants are susceptible to a systemic mottle type of infection.

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PATHOGENIC DIFFERENCES MANIFESTED BY ERWINIA ATROSEPTICA AND ERWINIA CAROTOVORA[1]

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In recent publications Smith (5) and Burkholder and Smith (1) have demonstrated that *Erwinia atroseptica* and *E. carotovora* may be differentiated on cultural and biochemical characters. The separations formulated in these studies were considered complete enough for these pathogens to be considered two distinct species. If these cultural distinctions could be correlated with pathogenic manifestations within each group of isolates the species separation would become more apparent. The present article deals with the pathogenic phase of the problem.

The isolates used in these investigations are many of those listed and previously reported on in some detail (1), and consist of 32 isolates of *Erwinia atroseptica* and 24 of *E. carotovora*. Reference to the above article will give the source and history of the isolates studied in this paper. All of them produced a soft rot of potato slices. At times there was a slight variation in the type of symptom produced on the slices, but these differences were inconsistent and of no value in separating the two species. Furthermore, the host range of the two pathogens as soft rot producers (1), when artificial inoculations were made on 15 different vegetables, was the same. The slight differences shown between the two pathogens, in this type of study, cannot be considered great enough for specific differentiation.

Distinct differences, however, were shown between the two species when inoculations were made at the base of young growing potato plants. The Sebago variety was used in all the inoculation experiments. The results of these inoculations, as previously reported (1), show that no one of the 24 isolates of *Erwinia carotovora* tested produced blackleg or wilt of young potato plants. In comparison, 23 of the 31 isolates of *E. atroseptica* tested produced a wilt of the leaves and black discoloration of the stems. Records on these plants were made from 4 days to a week after inoculation, at which time blackleg symptoms were very pronounced. Plants that did not show blackleg or wilt within a week did not develop symptoms later, although such plants were sometimes held for several weeks.

The biochemical reactions of the eight isolates that failed to produce blackleg were identical with reactions of *Erwinia atroseptica*, but they had been on artificial media for many years and probably had lost certain aspects of their virulence. At least two of these cultures had 10 years

[1] Excerpt from thesis presented July 1948 to the faculty of the Graduate School of Cornell University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer expresses his appreciation to Dr. W. H. Burkholder and F. M. Blodgett for their advice and assistance readily given during the course of these studies.

previously been shown by Waldee (6), and earlier by Jennison (2), to produce blackleg of growing potato plants. The fact that this property was lost by these two cultures indicates that it also could have been lost by the other six cultures. On the other hand, even very recent isolates of *E. carotovora* did not produce blackleg of young plants. This suggests that with this species the nonproduction of symptoms was not due to loss of virulence.

A further set of growing plant inoculations not previously reported with these two pathogens, and termed "top inoculations," is described below.

Sebago potato plants, approximately 1 ft. high, were inoculated by removing the terminal bud and immediately inverting a block of agar from a 48-hr. slant culture on the freshly cut surface of the stem. Agar blocks containing no bacteria were placed on the freshly cut stems of other plants to serve as checks. The major portion of the stem at this time was in a soft, succulent condition. After inoculation the plants were placed in a greenhouse at 70° F. If inoculations were made on clear warm days the plants were shaded with moist cheesecloth to prevent rapid drying of the agar blocks. On cloudy or rainy days this shading was not necessary.

All of the cultures of *Erwinia atroseptica* and *E. carotovora*, totaling approximately half of each group of isolates, tested in these experiments, caused infection of the tops of the stems. The symptoms consisted of a black discoloration, and wet disintegration of the stems. The degree of infection varied with the different isolates, from an infection extending $\frac{1}{2}$ in. below the inoculation point to an infection consuming the entire stem. The cultures that produced blackleg when inoculated at the base of the stems, as a general rule, caused a more extensive infection when inoculated at the top of the stems. Black discoloration of the infected zone was apparent with all of the cultures tested, and though the degree of discoloration varied it could not be considered a distinctive character.

An additional symptom was noted in plants inoculated in this manner. Plants inoculated with cultures of *Erwinia atroseptica* that produced blackleg showed decided epinasty of the leaf petioles. In some plants this symptom developed at a considerable distance below the black discolored area of apparent infection. In others, the epinasty of the leaves persisted though infection had consumed most of the stem. The more customary change from epinasty to wilting therefore was not observed in all instances. Although epinasty was also noted in plants inoculated with *E. carotovora*, it was not so pronounced nor did it occur so often as in the case of *E. atroseptica*. The differences in pathogenic reactions of the two species with this method of inoculation are not so distinct as with base inoculations, but at least slight pathogenic variation has been shown to exist between the two pathogens.

CUTTING-KNIFE TRANSMISSION

It has been shown above that the blackleg pathogen *Erwinia atroseptica* differs from the soft-rot pathogen *E. carotovora* in its pathogenic manifestations on artificially inoculated growing plants. As both of these organisms

have been reported to cause blackleg symptoms and seed-piece rot, it was considered appropriate that the two pathogens should be tested in cutting-knife inoculation experiments. Transmission of the blackleg pathogen on a cutting knife and subsequent seed-piece infection was suggested by Orton (4) in 1917, but he presented no data which would show this to be true.

For the cutting-knife experiments, diseased tubers were obtained by injecting healthy tubers with a water suspension from pure cultures of *Erwinia atroseptica* with a hypodermic syringe. The suspension was prepared by washing the bacteria from a 48-hour-old slant culture with sterile distilled water, and the inoculated tubers were incubated in moist chambers at room temperature for 48 hr. A similar series of tubers was inoculated with *E. carotovora*. Tubers which developed a considerable decayed area were used as inoculum in the cutting-knife transmission studies. Naturally infected tubers were not used in these experiments, since such tubers may contain one or both, or additional organisms, and a strict comparison between the two species could not be made.

A sterilized knife was infested with bacteria by cutting the tubers which had developed soft rot. This infested knife then was used to cut in succession three seed pieces from healthy tubers. Since the knife was not recontaminated between successive cuts, the bacterial population on the knife was reduced with each cut. Seed pieces cut with a sterilized knife served as checks. The seed pieces from the original inoculated tubers, those inoculated with the cutting knife, and the checks were planted immediately in steamed soil in 4-in. pots in the greenhouse.

TABLE 1.—*Development of sprouts and blackleg symptoms after potato tubers were inoculated with four isolates of Erwinia atroseptica and four of E. carotovora*

Experiment No.	Hypodermic inoculations			Cutting-knife inoculations								
				First cut			Second cut			Third cut		
	P.[a]	S.	Bl.	P.	S.	Bl.	P.	S.	Bl.	P.	S.	Bl.
						Erwinia atroseptica						
1	50	4	0	26	2	0	26	23	3	21	19	3
2	30	0	0	30	8	1	30	12	2	30	24	0
3	30	11	2	30	30	0	30	30	0	30	30	0
4	40	14	1	40	23	3	40	37	3	40	40	0
5	30	3	1	30	20	1	30	22	3	30	25	5
Totals	180	32	4	156	83	5	156	124	11	151	138	8
Percentages		18	13		53	6		80	9		91	6
						Erwinia carotovora						
1	45	43	0	23	23	0	22	22	0	20	19	0
2	30	21	0	30	29	0	30	28	0	30	27	0
3	30	30	0	30	28	0	30	30	0	30	29	0
4	40	37	0	40	37	0	39	33	0	40	38	0
5	30	26	0	30	29	0	30	30	0	30	30	0
Totals	175	157	0	153	146	0	151	143	0	150	143	0
Percentages		90	0		95	0		95	0		95	0

[a] P = number of seed pieces planted; S = number of seed pieces sprouted; Bl = number of seed pieces producing blackleg plants.

At various intervals throughout the duration of the experiments records were taken on the number of seed pieces developing sprouts, and the number of plants showing blackleg symptoms. In these studies four isolates each of *Erwinia atroseptica* and of *E. carotovora* were used. It was thought by using several strains of each species, peculiarities of a particular strain would be overcome, and if differences between the two species could be demonstrated the distinctions would be more conclusive. The experiment was repeated five times and a summary of the data obtained is presented in table 1. Experiments 1, 2, and 5 were with tubers that had been in storage at 40° F. for several months and had formed long sprouts on exposure to warmer temperature. Experiments 3 and 4 were with tubers that had been in the same storage chamber for a shorter period, and though they sprouted readily at warmer temperatures, the sprouts were short and the tubers remained firm.

If the five experiments are totaled, these data show that of 180 seed pieces planted from the original tubers inoculated by means of a hypodermic syringe with *Erwinia atroseptica*, only 32 or 17.8 per cent developed sprouts above the soil line. Of these 32 plants 4, or 12.5 per cent, later developed blackleg symptoms. In the transmission to healthy seed pieces not only was there a decrease in stand which became less pronounced with each successive cut, but blackleg plants occurred throughout. A 74.5 per cent stand was obtained from 463 seed pieces involved in the transmission studies. Twenty-four or 6.9 per cent of these plants later developed blackleg symptoms. The results of these experiments also indicate that with old tubers, as found in experiments 1, 2, and 5, the loss attributed to this pathogen is apt to be greater than with tubers that have only recently broken dormancy. Tubers of the former class are often used as planting stock in many areas of New York State.

Decidedly different results were obtained when tubers were inoculated with *Erwinia carotovora*. When seed pieces from the tubers inoculated with the hypodermic syringe were planted, 157 out of a possible 175 developed sprouts above the soil line, or a stand of 89.7 per cent. Of the 454 seed pieces that had been inoculated with a cutting knife, 432 or 96.4 per cent developed sprouts above the soil line. Infection with this organism, therefore, apparently does not result from transmission on a cutting knife. Also with *E. carotovora* the difference in age of the inoculated tubers had little or no effect on the resulting stand. In no case was blackleg produced by this organism. The stand resulting from tubers inoculated with *E. carotovora* compares favorably with the stand of the uninoculated check seed pieces. In the latter case, of 150 seed pieces planted, 139 developed sprouts above the soil line for 93 per cent stand, and no blackleg symptoms were produced.

In 1926 Leach (3) suggested that the cork barrier formed by a potato is sufficient to prevent the spread of a soft-rot organism throughout the tuber. It is possible that this barrier is sufficient to prevent the spread

of *Erwinia carotovora* throughout the seed pieces, but was not sufficient to prevent the spread of *E. atroseptica*.

In order to test whether cork formation prevented either of these organisms from attacking a tuber, a series of experiments was conducted. Slices of Sebago potatoes were placed in moist chambers with moist filter paper to keep the humidity high, and the chambers placed in constant temperature incubators. Slices remained at temperatures of 15° and 21° C. for 2, 3, 4, and 5 days. The slices were then inoculated by spreading a thin layer of bacteria from a 48-hr. slant culture on the surface of the slice. Fourteen different isolates of *Erwinia atroseptica* and 15 isolates of *E. carotovora* were tested. The slices were then returned to their respective incubators.

The data obtained from these experiments (Table 2) indicate that the

TABLE 2.—*Cork penetration by 14 isolates of Erwinia atroseptica and 15 isolates of E. carotovora*

Corking		*E. carotovora*			*E. atroseptica*		
			Penetrations			Penetrations	
Number of days	Temperature	Number of slices	Number	Per cent	Number of slices	Number	Per cent
2	15° C.	15	9	60	15	13	87
2	21° C.	15	6	40	15	15	100
3	15° C.	28	10	36	28	24	86
3	21° C.	43	4	9	36	28	78
4	15° C.	45	5	11	45	38	84
4	21° C.	45	3	7	45	22	49
5	15° C.	14	3	21	14	11	79
5	21° C.	14	1	7	14	8	57

penetration of the protective barrier takes place more often with *Erwinia atroseptica* isolates than with *E. carotovora* isolates. The data also show that, with the exception of those slices held only 2 days, before inoculation the protective barrier forms more rapidly at 21° C. than at 15° C., as indicated by the decreased percentage of penetration at the higher temperature. The fact that even after the slices had corked for 5 days at 21° C. 57 per cent of them were penetrated by *E. atroseptica* would show that this particular pathogen has the ability to penetrate well formed cork layers.

If a comparison is made between these data and the data on cutting-knife transmission, at least a partial explanation can be obtained for the reduction in stand caused by the blackleg pathogen and not by *Erwinia carotovora*. The freshly cut seed pieces in the cutting-knife experiment were planted in soil presumably slightly below 21° C. (air temperature approximately 70° F.), and it is shown in the cork penetration experiments that at this temperature *E. atroseptica* has the ability to penetrate the cork barrier. Freehand sections made in the cork penetration studies showed the infected area of the slice was walled off by a protective layer. At points in this barrier the bacteria had broken through, and the decay progressed in this manner until the slice was decomposed. This may have happened in the

cutting-knife experiments in many of the seed pieces inoculated with *E. atroseptica*, but not in the case of *E. carotovora*.

DISCUSSION AND CONCLUSIONS

The pathogenic manifestations of 56 isolates of *Erwinia atroseptica* and *E. carotovora* have been studied as a basis of separating the two pathogens. A close correlation was found to exist between the physiological and pathological differences of the two species. Only those isolates classified as *E. atroseptica*, on the basis of physiological data, produced blackleg when young potato plants were artificially inoculated at the base of the stem. When the plants were inoculated in the fleshy, succulent tissue at the top of the stems, the differences between species were not so definite. This tissue may be compared with the fleshy tissue of many vegetables commonly attacked by *E. carotovora*, and differs from older and more differentiated tissue of the lower portion of the stem which in these experiments was invaded only by *E. atroseptica*. From the data presented it is concluded that the blackleg pathogen is more aggressive on potato plants than *E. carotovora*, and the two pathogens should be considered distinct species.

The same distinction was found between the two species in the cutting-knife inoculation studies. When seed pieces were inoculated with *Erwinia atroseptica* a rot of the seed piece and a resulting decrease in stand occurred. Many of the plants that developed from the inoculated seed pieces showed blackleg symptoms. The seed pieces inoculated with *E. carotovora* showed a good stand and none of the plants developed blackleg symptoms. It has been suggested, on the basis of cork penetration studies, that the lack of seed-piece decay in these experiments may be due to the inability of *E. carotovora* to penetrate the protective barrier formed by the seed piece. The results of these experiments indicate that *E. atroseptica* is the more common cause of bacterial seed-piece decay so often found in the field, and that *E. carotovora* alone rarely causes severe seed-piece decay. The decay of seed pieces often attributed to *E. carotovora*, therefore, may be the result of infection of several soft-rot organisms. The fact that in these experiments blackleg symptoms were produced only by *E. atroseptica* indicates that this pathogen may be the sole cause of this disease under field conditions.

SUMMARY

Erwinia atroseptica causes a soft rot of potato tubers, and blackleg symptoms on growing plants.

Erwinia carotovora causes a soft rot of potato tubers, but does not cause blackleg symptoms on growing plants.

Erwinia atroseptica may be transmitted from diseased to healthy tubers on a knife used in cutting seed pieces. Seed-piece infection results in decrease in stand and development of blackleg symptoms.

Under the conditions of these experiments no infection of the potato

plants resulted when seed pieces were inoculated with *Erwinia carotovora* by means of a cutting knife.

A close positive correlation exists between the separation of soft-rot isolates by biochemical reactions, and the separation of the same isolates by pathological reactions.

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ZONATE CANKER, A VIRUS DISEASE OF AMERICAN ELM

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(Accepted for publication April 12, 1950)

In 1941 an unusually severe case of elm mosaic was observed in Lawrenceville, New Jersey, on a large specimen of *Ulmus americana*. Since the virus seemed to be exceptionally virulent, bark patches were secured from the tree in June 1941 and grafted to approximately 5-year-old healthy nursery elms for comparison of the disease with other cases of mosaic which had been observed previously.[2] Mottle symptoms of elm mosaic were obtained the following year, and two of the grafted trees also developed necrotic leaf spots shortly after leaf emergence in early spring. Later the necrotic spotted leaves were shed. New leaves that developed were small with symptoms of mottle and rugose mosaic, but without necrotic spotting. In late summer, these two trees developed small necrotic cankers in the bark of their upper stems and branches. The cankers enlarged and extended into the cambium region, sometimes resulting in girdling of affected branches and death of their terminal portions. Beneath the surface of larger cankered areas, light and dark brown zones or concentric rings occurred in the necrotic bark tissue.

Bark patches were secured from the margins of cankers of one of the affected nursery trees in the spring of 1943 and grafted to the lower stems of three healthy nursery elms. Tops of the three grafted trees and two ungrafted checks in this experiment, all 3-year-old trees, were severely pruned about 2 weeks after the grafts were made. On two of these grafted trees brown necrotic spots appeared by early summer, on some leaves of the new growth. Succulent lateral sprouts bearing necrotic spotted leaves on these two trees wilted and died, and typical zonate cankers developed in the bark of the main stem and branches, frequently centering on the lateral sprouts which had died. The other grafted tree and the two ungrafted checks remained healthy. By fall of 1943, all trees affected by the zonate canker had died.

In 1947, zonate canker was again observed in a group of trees in an experiment on the elm phloem necrosis virus.[3] The experiment had been designed to determine how long the phloem necrosis virus might be present

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[2] Swingle, R. U., P. E. Tilford, and C. F. Irish. A graft transmissible mosaic of American elm. Phytopath. 33: 1196–1200. 1943.

[3] Swingle, R. U. Phloem necrosis, a virus disease of the American elm. U. S. Dept. Agr. Cir. 640. 1942.

in large shade-tree elms before disease symptoms could be recognized in infected trees. In this experiment, 20 branch specimens, $\frac{1}{4}$ to $\frac{1}{2}$ in. in diameter, were collected at monthly intervals from each of 15 large, apparently healthy elms in Marietta, Ohio. A bark patch was removed from each branch specimen and grafted to the lower stem of a healthy 3-year-old nursery tree. A total of 1146 healthy nursery elms were grafted in this manner during the spring and summer of 1946. In 1947, 46.6 per cent of

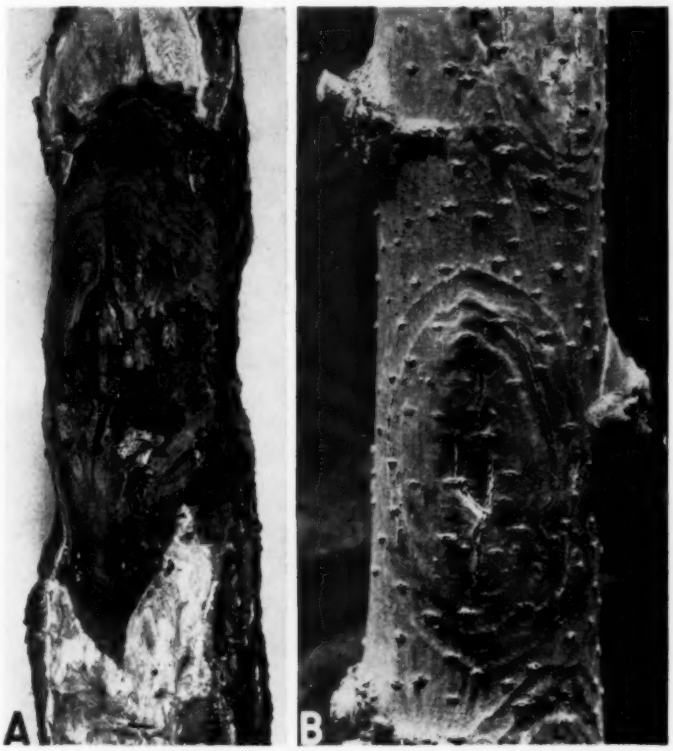

FIG. 1. A. Elm stem with rough, outer bark removed to show internal symptoms of a zonate canker at the point of grafting with a diseased bark patch. B. Zonate canker on the upper stem of a grafted tree.

these grafted trees had mild to severe symptoms of elm mosaic, mostly of the mottle and rugose types. In addition to the mosaic symptoms, some of the grafted trees developed cankers at the point of grafting and at irregular intervals on their upper stems and branches. The cankers appeared on trees grafted with bark patches from seven of the 15 apparently healthy elms, and the number of trees thus affected varied from 5 to 55 per cent, depending upon which of the seven trees had supplied the bark patches used. When the outer bark was shaved from the cankered areas, concentric rings or zones of living and dying or light and dark brown dead tissue were exposed (Fig. 1, A). Occasionally, concentric rings were visible on the bark surface of young branches (Fig. 1, B). The zonate cankers first appeared

in the bark as rings of dead and living tissue in the cortical or phloem tissue. Later the cankers enlarged, the centers of the cankers consisting of light and dark brown concentric rings of dead tissue that extended to the xylem and rings of living and dead or dying tissue at the canker margins. The symptoms suggested the diffusion of a lethal substance through the bark tissue in a manner similar to the Liesegang phenomenon.[4] Frequently stems and branches were girdled and their upper portions died. In none of

FIG. 2. Necrotic leaf spots associated with the zonate canker disease.

the cases observed did cankers develop on the stem below the point of grafting. However, epicormic branches often developed from the main stem below the graft and frequently brown, necrotic spots about 2 cm. in diameter developed on their leaves. On a single leaf these necrotic spots were usually few in number, but sometimes numerous spots coalesced to involve a large portion of the leaf (Fig. 2).

Reexamination of the 15 apparently healthy trees at Marietta, Ohio, from which the grafting material had been obtained, revealed no foliage or bark symptoms of either mosaic or zonate canker. Some of these trees were of poor vigor, but this was not a consistent characteristic.

[4] Liesegang, R. E. Über einige Eigenschaften von Gallerten. Naturwiss. Wochenschr. 11: 353–362. 1896.

Although the cases of zonate canker observed were confined to grafted trees and seemed to be of virus nature, the isolation of a causal organism was attempted by making diseased tissue plantings on potato-dextrose agar. Most of the attempted cultures from marginal areas of cankers remained sterile, and organisms obtained from dead inner portions of the canker did not prove to be pathogenic. Also, microscopical examination of the necrotic spots on leaves failed to reveal any organism consistently present either on or within the necrotic tissues.

A series of further experiments on transmission of the zonate-canker disease was undertaken on June 16, 1948, on healthy, 3-year-old *Ulmus americana* nursery stock.

In one of these experiments, bark patches taken from the margins of zonate cankers on six diseased trees were grafted to the lower stems of 17 healthy elms. The tops of these grafted trees and eight ungrafted checks were severely pruned 10 days after the grafts were made. The first case of zonate canker was observed on a grafted tree around the point of grafting on July 30, 1948. In the spring of 1949, necrotic leaf spots were observed on six of the grafted trees, and by November 1949, eight of the 17 grafted trees had developed zonate cankers around the points of grafting and on their upper stems and branches.

In a second experiment, bark patches were removed from the lower stems of eight healthy trees, their inner surfaces were rubbed against the inner surfaces of diseased bark patches with carborundum as an abrasive, and the healthy patches were then returned to the healthy trees. None of the healthy trees had developed necrotic leaf spotting or other symptoms of zonate canker by November 10, 1949.

In a third experiment, thin slices of bark from zonate cankers were placed in slanting stem wounds that extended into the wood of three healthy elms. The wounds were then taped with rubber grafting bands. By November 1949, all three of these trees had developed symptoms of zonate canker.

In a fourth experiment, areas on the stems of four healthy trees were shaved to expose the phloem tissue. The exposed phloem was covered with diseased bark patches and tied with rubber grafting bands. Three of the four trees had developed symptoms of zonate canker by November 1949.

In the last experiment of this series, necrotic spotted leaves and diseased bark from zonate canker trees were macerated by mortar and pestle with just enough distilled water to produce a thick paste. The macerated tissues were placed in a cheesecloth bag. Expressed juice was wiped onto six leaves on each of 20 healthy elms, with fine carborundum powder as an abrasive. None of these 20 trees had developed symptoms of zonate canker by November 1949.

In June 1948, a tree of *U. americana* was observed at Columbia, Missouri, that appeared normal with the exception of necrotic leaf spots that seemed

to be identical with those associated with zonate canker. Bark patches from branches of this tree were grafted to four 3- to 4-year-old healthy American elms. Necrotic leaf spots, necrotic bark patches, and zonate cankers appeared on two of the trees by the fall of 1948. These trees were dead in the spring of 1949. In May 1949, five additional, approximately 3-year-old healthy elms were grafted with material from the field specimen showing necrotic leaf spotting, and by the fall of 1949, two of these trees had developed zonate cankers and the necrotic leaf-spot symptom.

SUMMARY

Zonate canker is a previously undescribed disease of *Ulmus americana* that is transmissible by grafting and apparently of virus nature. Attempted transmission of the zonate canker virus by leaf inoculations failed, but some transmission attempts other than by common grafting techniques were successful. In the latter tests, the possibility of graft union between diseased and healthy tissue was not precluded. The zonate canker virus has been found to occur in trees in New Jersey, Ohio, and Missouri. It has been found alone and in combination with the elm mosaic virus. Both mosaic and zonate canker have been found to occur as latent viruses in large shade-tree elms.

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CERCOSPORA LEAF SPOT OF TALL FESCUE AND SMOOTH BROME[1]

MARVIN D. WHITEHEAD AND E. C. HOLT[2]

(Accepted for publication April 6, 1950)

Cercospora leaf spot incited by *Cercospora festucae* was first described by Hardison[3] from tall fescue, *Festuca elatior* var. *arundinacea* (Schreb.) Shimm, collected in Kentucky in July, 1944. He stated that "the effect of the disease has thus far been mild." No other report of the disease has since appeared. The leaf spot occurred in severe proportions on tall fescue and smooth brome, *Bromus inermis* Leyss, in the grass nurseries at College Station and Temple, Texas, during the summer of 1949. Stand counts made at College Station on November 7 revealed that approximately 10 per cent of 1-year-old seedlings of the Kentucky 31 strain of tall fescue and 30 per cent of the Alta strain had been killed by the disease. In a spaced planting of mature clones of Alta fescue 100 per cent of the clones were severely infected and 36 per cent were either dead or had fewer than 10 living tillers. No mature clonal plantings of smooth brome were killed by the disease although approximately 50 per cent were severely infected and produced a markedly reduced amount of inferior forage.

The leaf spot was first noted in April, and more severe symptoms developed as the summer progressed. The disease began to subside with the occurrence of the fall rains during October, although infections continued to appear on the vigorous new leaves.

The organism attacks the foliage of tall fescue causing oval to elongate leaf spots 0.5–8 mm. in length with gray centers and purple to purple-brown borders (Fig. 1, A and B). The initial infections occur on the leaf tips, later spreading down the leaf. The spots coalesce, inciting tip necrosis. On the foliage of smooth brome the spots are more cylindrical, commonly smaller and more numerous, causing early tip necrosis and imparting a general gray-black color (Fig. 1, C).

Johnson and Valleau,[4] from cross inoculation studies on tobacco, have placed *Cercospora festucae* in synonymy with *C. apii* Fr. As the present collections indicate that the original description was incomplete, drawings of the fungus (Fig. 2) and an amended description are presented to record

[1] Published with the approval of the Director as Technical Article No. 1270, Texas Agricultural Experiment Station.

[2] E. C. Holt, Department of Agronomy, Texas Agricultural Experiment Station.

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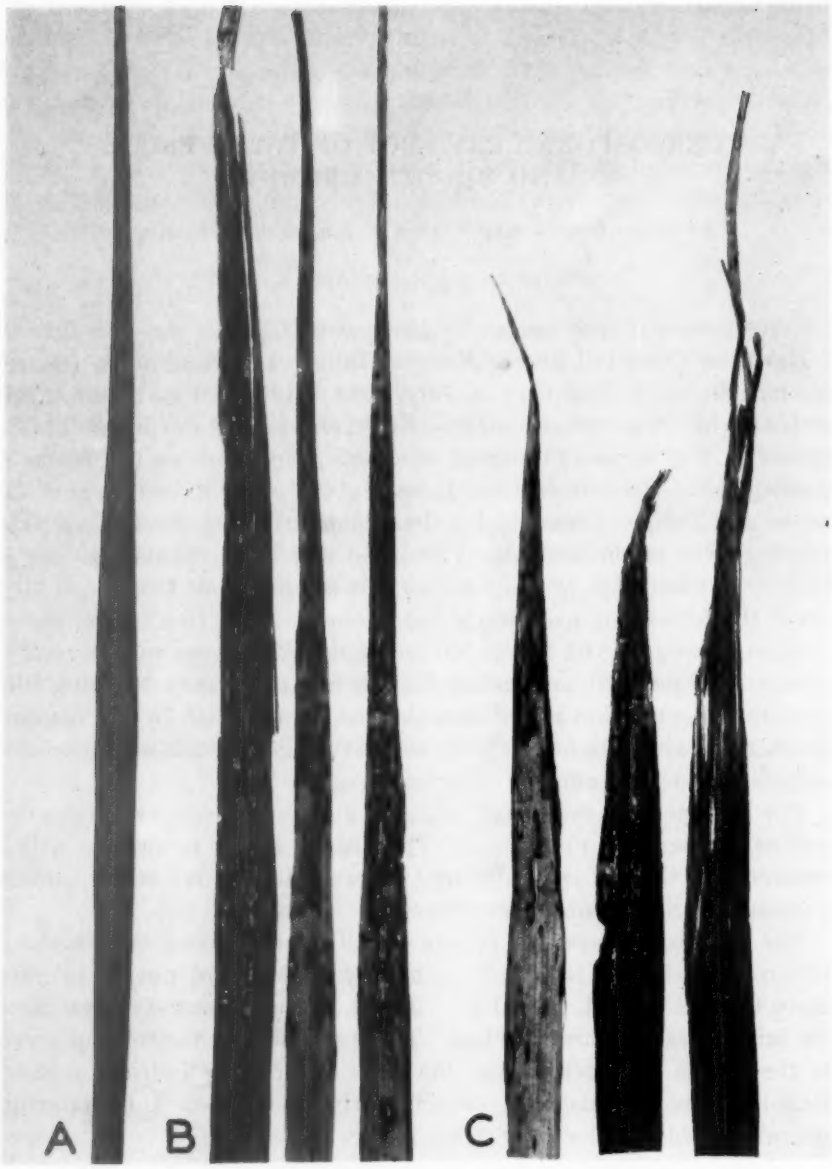

FIG. 1. Symptoms on leaves infected with *Cercospora festucae* (×4/5): A, initial infections at tip of fescue leaf; B, severely infected fescue leaves; C, severely infected brome leaves.

the morphology of the fungus as it appears on these two economically important grasses.

AMENDED DESCRIPTION[5]

Cercospora festucae Hardison.

Maculae in foliis *Festucae elatioris* v. *arundinaceae* ovales usque elongatae, 0.5–8 mm. longae, centralibus griseis et marginibus purpureis vel purpureo-brunneis, primum

in apicibus foliorum, demum se extendentes et coalescentes, in foliis *Bromi inermis* cylindraceae, plerumque minores pluresque, necrosem apicalem et colorem griseo-atrum efficientes; conidiophora ad bases olivaceo-brunnea, ad apices pallidioria et subattenuata, 3–5 × 39.4–800 μ (medio 354.9 μ) longa, 0–18- (medio 6-) septata, longiores 1–2 geniculata, fere recta, apicibus rotundatis et truncatis, typice eramosa sed interdum ramosa, singulatim vel in caespitibus caulium 2–32 (medio 12.9), plerumque e stomatibus, interdum e cellulis parvis connectentibus orientia; stromata carentia vel cellulis paucis brun-

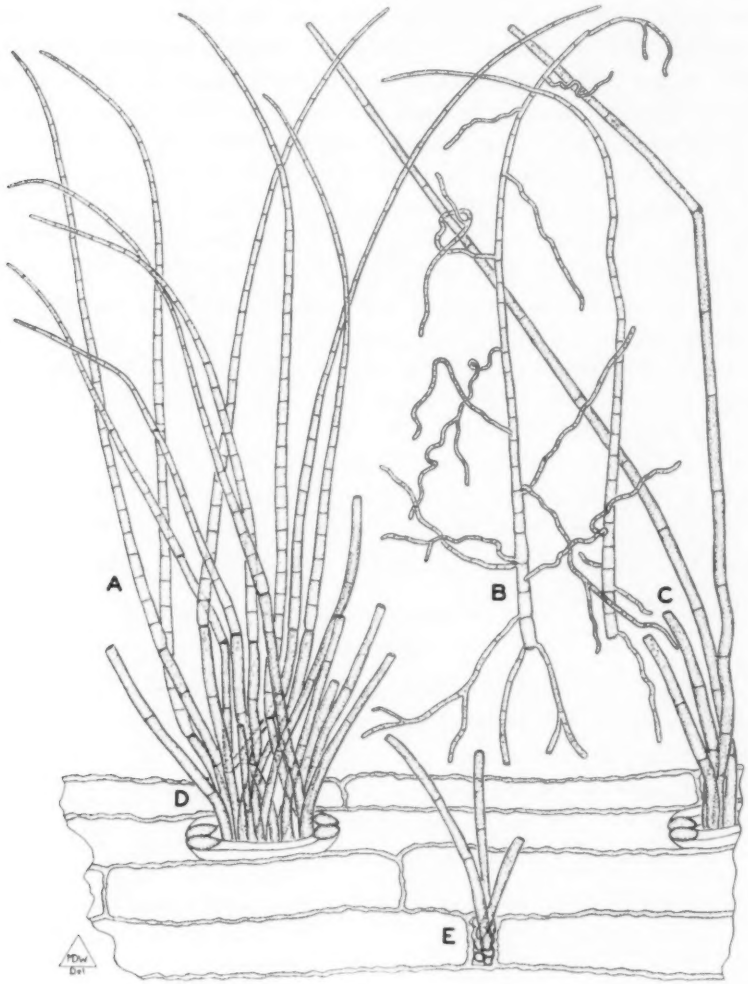

FIG. 2. Morphological characters of *Cercospora festucae* (×610): A, conidia; B, germinating conidia; C, long conidiophores, one showing geniculation; D, group of conidiophores arising from stoma; E, group of conidiophores arising from small connecting cell.

neis consistentia; conidia hyalina, acicularia, in maturitate curvata, 4–32- (medio 19-) septata, ad basim truncata, apicem rotundatum versus attenuata, 1.6–4.9 × 40–361.5 μ (medio 214.7 μ) longa, e cellula aliqua germinantia.

[5] The authors are indebted to Dr. Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, U. S. Dept. Agr., for the preparation of the Latin diagnosis.

Hab. parasitica in foliis *Festucae elatioris* var. *arundinaceae* et Bromi inermis, Texas.

Attacks the foliage of *Festuca elatior* var. *arundinacea* where it causes oval to elongate spots 0.5–8 mm. in length with gray centers and purple to purple-brown borders. First occurring on leaf tips, later spreading down the leaf, spots coalesce inciting tip necrosis. On the foliage of *Bromus inermis* spots are more cylindrical, commonly smaller and more numerous, inciting early tip necrosis and imparting a general gray-black color. Conidiophores are pale to medium olivaceous-brown near the base, paler and sometimes tapering slightly toward the tip, 3–5 × 39.4–800 μ (mean 354.9) long, 0–18- (mean 6-) septate, longer conidiophores 1–2 geniculate, almost straight, with rounded truncate tip, typically not branched, occasionally branching, arising singly or in groups of 2–32 (mean 12.9) divergent stalks, usually from stomata, occasionally from small connecting cells, stromata none or a few brown cells. Conidia are hyaline, acicular, curved when mature, 4–32 (mean 19) septate, base truncate tapering to a rounded tip 1.6–4.9 × 40–361.5 μ (mean 214.7) long, germinating from any cell.

Habitat: Parasitic on *Festuca elatior* var. *arundinacea* and *Bromus inermis* collected at College Station, Texas, October 1949; and Temple, Texas, October 1949.

Portions of the collection were deposited in the mycological herbarium of the U. S. Department of Agriculture, Bureau of Plant Industry, Beltsville, Maryland; New York Botanical Garden, Bronx, New York; Harvard University, Cambridge, Massachusetts; and Texas A. & M. College herbarium, College Station, Texas.

SUMMARY

Cercospora festucae Hardison is reported to cause a severe leaf spot of tall fescue and smooth brome grass. Amended description and drawings of the organism are presented.

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RAMULARIA CYCLAMINICOLA TREL., THE CAUSE OF CYCLAMEN STUNT DISEASE

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(Accepted for publication April 24, 1950)

Two phases of the same disease sometimes are attributed to different pathogens. This appears to have been the case with stunt and the Ramularia leaf disease of cyclamen (*Cyclamen indicum* L.). This paper reports observations and studies showing that Ramularia leaf disease, stunt, and a previously undescribed wilt are caused by a single organism, *Ramularia cyclaminicola* Trel.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Ramularia leaf disease of this crop was observed by Trelease (12) in Illinois in January, 1914. He attributed the disease, without inoculation tests, to a fungus which he described as *Ramularia cyclaminicola*. Older leaves of infected plants wilted at time of flowering, and areas on lower leaf surfaces near the soil developed a frosty appearance where the Ramularia was sporulating. What appears to have been the same disease occurred in Minneapolis, Minnesota, on September 17, 1924 (J. L. Seal. Mycological Collections, Bureau of Plant Industry). It has also been reported from Ohio.[1] *Ramularia cyclaminicola* was observed by us on basal leaves of wilted cyclamen plants in a Los Angeles greenhouse on November 8, 1946.[2] We also found the fungus on flaccid, yellow, basal leaves (Fig. 1, A) of typical stunt plants from Natick, Massachusetts, on November 18, 1948.[3] The invaded areas had indefinite diffuse margins and were similar to lesions produced on narcissus leaves by *R. vallisumbrosae* Cav. (3) and on sweet pea by *R. deusta* (Fckl.) K. F. Baker, Snyder, and L. H. Davis (1).

The stunt disease was first found in New York in 1926 and in Ohio in 1929 by Massey and Tilford (8). They also reported it in New Jersey, Pennsylvania, and California. Plants characteristically were stunted, with flowers remaining below the small, sometimes yellow leaf blades. In cut corms reddish-brown necrotic areas were seen; these areas were particularly evident at the crown but also occurred in petioles, peduncles, and large roots. These workers isolated a slow-growing fungus from affected tissue and named it *Cladosporium cyclaminis* Massey & Tilford, basing the

[1] Letter from Dr. Freeman Weiss, November 12, 1948.

[2] Specimen in Herbarium, Division of Plant Pathology, University of California, Los Angeles.

[3] Accession No. 37799, Herbarium, Department of Plant Pathology, Cornell University.

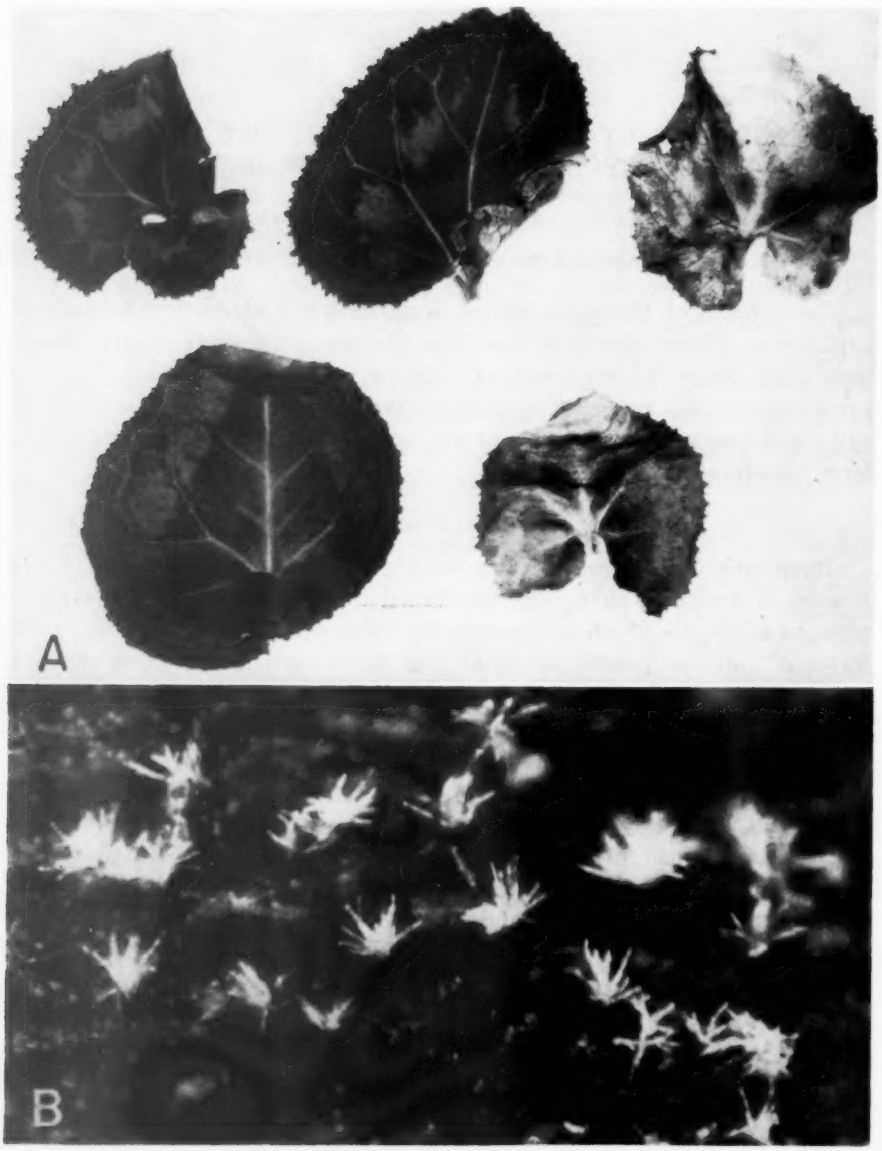

FIG. 1. A, leaves from stunt cyclamen, showing lesions on which sporulation of *Ramularia cyclaminicola* developed; Natick, Massachusetts, November 1948. B, enlargement of conidial fascicles produced on lower surface of cyclamen leaf inoculated with a pure culture of the stunt fungus; Los Angeles, California, September, 1949.

description on morphology in culture, since no sporulation was observed on the host. Symptoms of the disease are well illustrated by Tilford (11, Figs. 7 and 8) and Smith (10, Fig. 5). The stunt disease was also reported in Montreal, Quebec, in 1941 (5) and 1942 (6). Typical specimens were sent to us by Dr. Stephen Wilhelm from Denver, Colorado, in Novem-

ber 1944.[2] The disease is widely distributed in North America, commonly causing moderate losses, but sometimes affecting the entire crop.

Apparently neither the disease nor the fungus has been reported outside of North America. The cyclamen, native to the eastern Mediterranean region, was introduced into Europe and greatly improved. The plant was imported into America from Germany and Holland, first as dormant corms and later as seeds. Because leadership in development of this crop has been retained by the Dutch and Germans, there probably has been little shipment of plants or seed from North America. If stunt, Ramularia leaf disease, or wilt occurred in Europe, it would have been reported in the various papers published there on cyclamen diseases. It is probable, therefore, that *Ramularia cyclaminicola* is native to North America, perhaps on one of the several other members of the Primulaceae on which Ramularias have been reported.

Mes[4] studied a disease complex of cyclamen that was severe in the San Francisco area in 1930, but said to have been present throughout California for about 10 years. Photographs of plants and of cut corms indicate that some typical stunt must have been involved. The organism isolated was identified as a *Ramularia*. Since this fungus produced colonies 9 cm. in diameter in 15 days at 25° C., it is improbable that it was a *Ramularia*, as members of that genus grow slowly in culture (1). Dr. H. N. Hansen, who saw these cultures, states that the organism probably was a *Cylindrocarpon* sp., a common soil inhabitant in that area. There is no evidence that a *Ramularia* was observed on the foliage.

OBSERVATIONS AND CULTURE STUDIES

A commercial crop of flowering cyclamen observed in Los Angeles in November and December, 1946, showed severe wilting and yellowing of the basal leaves. Many or all of the flowers and upper leaves also were wilted. The cut corms had brown necrotic areas typical of stunt, but the flowers and leaves were not shortened. Because of these symptoms, it was at first suspected that the disease was caused by a vascular fungus such as *Fusarium* or *Verticillium*.

Examination of the under surfaces of the yellow, wilted, basal leaves during the prevailing moist weather revealed on some an inconspicuous, frosty fasciculate fungus (similar to figure 1, B). When grown in culture it was similar to *Cladosporium cyclaminis* from a typical stunt cyclamen, both in appearance and slowness of development, suggesting that the sporulation on the leaves might be the normal development of the *Cladosporium* on its host. Microscopic examination showed that the fungus on the leaves was a *Ramularia* that closely resembled *R. cyclaminicola*. It was known from earlier studies (1) that a fungus, named *C. album* Dowson because of its morphology in culture, also was a *Ramularia* as it developed in sweet-pea leaves.

[4] Mes, Margaret. A Ramularia disease of cyclamen. Unpublished manuscript, Division of Plant Pathology, University of California, Berkeley. 1932.

Isolations made from infected corms, roots, petioles, and peduncles of these wilted cyclamen plants consistently yielded a dark-green, slow-growing fungus entirely typical of other Ramularias we have studied. Single-spore cultures from the fungus on leaves were identical with the isolations from discolored corm tissue. Isolates from the corms of another lot of California cyclamen plants that had typical stunt, and single-spore cultures from sporulation on leaves of these plants, also were the same fungus. Single-spore cultures from the leaf fungus of plants with characteristic stunt in Massachusetts in 1948 again proved to be this *Ramularia*. Monospore transfers were made of all corm-tissue isolates from the stunted and the wilted California plants, and these were identical with the single-spore cultures from the leaves of both groups. It thus seemed probable that the Ramularia leaf disease, typical stunt, and the wilt symptoms were all produced by the same organism.

A culture of *Cladosporium cyclaminis* from New York stunt plants was kindly sent by Dr. L. M. Massey for comparison with the California pathogen. His fungus, which had been in culture for several years, developed colonies similar to ours except that a superficial white cottony growth appeared with age. After our isolates had been kept in culture for a year, they also developed this white growth, and were then identical with his cultures of *C. cyclaminis*. This superficial growth is a reversion to the mycelial (M) type of Hansen (4). Cultures of our isolates forwarded to Dr. Massey were regarded by him as closely similar to his fungus.

The minutely echinulate conidia of the California isolates germinate readily on water agar in 15 to 20 hr. (Fig. 2, C). On potato-dextrose agar white colonies 2 mm. across are produced in 92 hr. at about 25° C.; the fungus begins sporulation at this age, producing typical conidia (Fig. 2, G) and conidiophores (Fig. 2, D–F), some of which show a tendency toward fasciculation (Fig. 2, E), as they do on the host. After 5 days the colonies are 3 mm. in diameter, pale gray above and gray beneath, round and slightly heaped in the center; after 6 days they become 3–4.5 mm. in diameter and olive-green beneath; in 10 days the colonies are 7 mm. in diameter, round, distinctly heaped in the center, and gray-green with whitish margins. There are differences in appearance and morphology between a typical *Cladosporium* (*C. fulvum* Cke.; Fig. 2, L) and *R. cyclaminicola* (Fig. 2, D–G) in culture, as discussed elsewhere for *R. deusta* (1).

INOCULATION TESTS

Two types of inoculation studies were conducted. In one, cyclamen plants were inoculated with an isolate from leaf sporulation in order to determine whether stunt or wilt symptoms would be produced. In the second, leaves of cyclamen seedlings were inoculated with a corm isolate of the stunt fungus to determine whether typical Ramularia sporulation would result.

On February 3, 1947, potted cyclamen seedlings 1½–2 in. high were

inoculated with: (a) a monospore transfer of a culture from discolored corm tissue of a wilted cyclamen plant obtained in Los Angeles; (b) a single-conidial isolate from the sporulation on a yellow leaf of a wilted plant comparable to (a); (c) culture (supplied by Dr. L. M. Massey) from the discolored corm tissue of a typical stunt plant in New York. Colonies of each of these various isolates were removed from cultures on

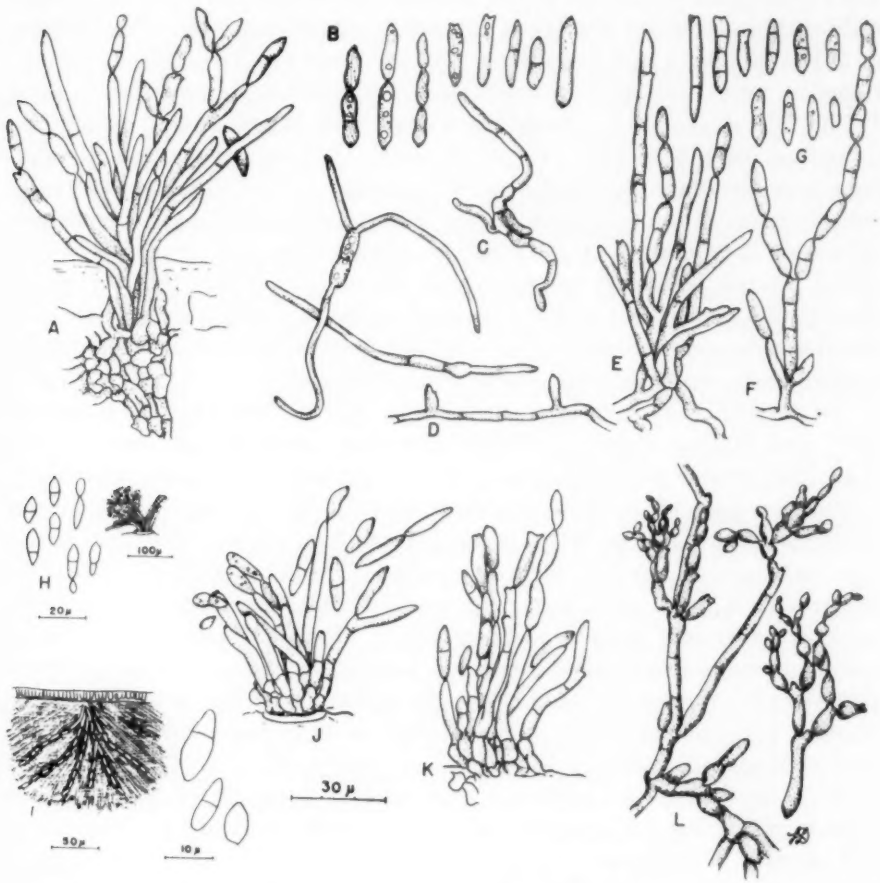

FIG. 2. Camera-lucida drawings of *Ramularia cyclaminicola* Trel. A, fascicle of conidiophores from cyclamen leaf collected in Los Angeles, November 1946. B, conidia from same collection. C, germinating conidia on water agar after 21 hr. D–F, conidiophore development in potato-dextrose-agar culture grown in light, showing tendency toward fasciculation (E). G, conidia developed in culture. H–I, tracings made from drawings by Trelease on herbarium sheet of type specimen. J, conidiophores and conidia from Trelease collection of Feb. 24, 1914 (Univ. Ill., Dept. Bot., Herb. No. 14247). K, conidiophores and conidia from type specimen (Univ. Ill., Dept. Bot., Herb. No. 14246.) L, *Cladosporium fulvum* from culture on potato-dextrose agar grown in light.

potato-dextrose agar and minced in sterile distilled water. Some of each of these conidial and mycelial suspensions was placed in the uninjured growing point at the apex of seedling corms, and some was painted on the upper and lower surfaces of uninjured leaves on other seedlings. There

were three plants in each series. The plants were placed in a large covered can, held in cool moisture-saturated air for 2 days, and then uncovered and moved to a glasshouse bench. Checks were handled in the same manner except that no inoculum was used; they were still healthy after 652 days. There were no successful inoculations evident in the series using Massey's New York stunt culture after 652 days, and it is thought that this was attributable to the age of the isolate. In the two series with younger California isolates, six plants out of nine developed stunt or wilt symptoms in 137 to 233 days, and the fungus was recovered from discolored vascular tissue in corms, and in some cases roots, of the four plants cultured after 137 to 323 days. Five plants were dead and therefore not culturable; these may have been killed by the fungus. A brief infestation of mealy bugs may also have contributed to their death. These data are limited in part because of our absence at the time plants developed the disease. However, they show that: (a) isolates from corms and from spores produced on the leaves of wilted plants are able to infect healthy tissue through the crowns and perhaps the leaves; (b) stunting, wilting, and corm discoloration were produced; (c) the original fungus was recovered from the infected corms; (d) the checks remained healthy.

In further tests mycelium and spores of the 1946 California isolates were used in June 1949 to inoculate unwounded crowns and leaves, and in July 1949 similar inoculum was placed in the wounded leaves of cyclamen seedlings. Lack of success with these, as with the old New York cultures mentioned above, when recent isolates proved infective, strongly suggests that pathogenicity is lost after prolonged growth in culture. The 1948 cultures from the typical stunt cyclamen plants from Massachusetts were used in additional tests in August 1949. Mycelium and spores free of culture medium were placed in wounds in the leaf blades of small seedlings that were held in moist chambers in the laboratory. The pathogen spread to other parts of the leaves, where flaccid yellow areas were produced and typical Ramularia sporulation developed (Fig. 1, B).

It is concluded as a result of these various types of evidence that the same organism causes common stunt, a previously undescribed wilt, and a leaf disease of cyclamen.

NOMENCLATURE OF PATHOGEN

To determine whether the fungus involved in these three phases of the disease in California and Massachusetts was the same as that described by Trelease in 1916, the type specimen was examined.[5] Comparison of the morphology of this specimen (Fig. 2, K) and a later topotype (Fig. 2, J) with the fungus causing leaf disease in California (Fig. 2, A and B) showed them to be the same. Sketches made by Trelease on the herbarium sheet (tracings in figure 2, H and I) show his concept of the species. The

[5] Univ. Ill., Dept. Bot., Herb. No. 14246. "*Ramularia cyclaminicola* on *Cyclamen persicum*. North Greenhouse, Univ. of Ill., Urbana: Jan. 14, 1914. Trelease." Loaned through the courtesy of Dr. Leland Shanor.

size, shape, and septation of the catenulate spores shown, and the fasciculate conidiophores, are the same as in his herbarium specimen and in the California material.

It is, therefore, concluded that the correct binomial for the fungus causing stunt, wilt, and leaf disease of cyclamen is *Ramularia cyclaminicola* Trel.

DISCUSSION

When the complete development of a member of the Fungi Imperfecti on its host and in culture is not known, its proper classification is difficult. For example, the fungus causing white blight of sweet pea, called *Cladosporium album* from growth in culture, should be named *Ramularia deusta*, based on sporulation on the host. When the causal organism of cyclamen stunt was described (8) in 1932, it was not known that this fungus sporulated on the leaves, and it was necessary to classify it on morphology in culture. Now that such sporulation has been seen, the binomial should be based on morphology on the host. *Cladosporium cyclaminis* Massey & Tilford thus becomes a synonym of the older *Ramularia cyclaminicola* Trel.

It is thought that this is the first report of a *Ramularia* that causes a leaf spot and is also associated with the vascular tissue of the shoot and roots. While this is an unusual occurrence for a fungus generally considered to be confined to leaf blades and stem cortex, it is not entirely unprecedented. *Peronospora parasitica* (Pers.) DeBary invades parenchyma of stems and roots of cauliflower, cabbage, and turnip (2, 7, 9). Conversely, some distinctly vascular pathogens are known to sporulate on the exterior of the host. This occurs with *Fusarium oxysporum* f. *callistephi* (Beach) Snyder & Hansen on aster, and a *Deuterophoma* sp. on chrysanthemum. *Verticillium albo-atrum* Reinke & Berth. was found by us sporulating on the basal leaves of a chrysanthemum with typical Verticillium wilt in Elmira, New York, on October 19, 1947.[6] The occurrence of a systemic or vascular pathogen among the cortical parasites of the genus Ramularia resembles the situation known in Fusarium.

Seed transmission of the cyclamen stunt fungus as mycelium in the integument has been demonstrated by Jacques (5, 6), who explained the dissemination of the pathogen in nature by this means. Tilford (11) and Smith (10) emphasized the importance of infested soil in such spread. With the observation that the fungus sporulates on its host, air-borne conidia must be considered as a third form of dissemination. It is unlikely that conidial infections of old plants could become damaging before the crop is sold, but seedling cyclamen infected by air-borne spores originating in the same glasshouse would have time to develop severe symptoms during the 12–18 months before sale.

Because of the possible importance of air-borne conidia in spreading the disease, some supplementary control procedures may be suggested. Seed-

[6] See footnote 2.

lings should not be grown in or near old infected plants. The use of a fungicidal spray might prove of benefit in protecting such seedlings, but there is question whether it would be warranted on older plants.

SUMMARY

Two known diseases of florists' cyclamen (Ramularia leaf disease and stunt), and another (wilt) described here, were found to be phases of a single trouble caused by *Ramularia cyclaminicola* Trel. *Cladosporium cyclaminis* Massey & Tilford becomes a synonym.

This disease complex apparently is limited to North America. The Ramularia leaf disease has been collected in Illinois, Minnesota, Ohio, California, and Massachusetts. Stunt has been reported in New York, Ohio, New Jersey, Pennsylvania, California, Quebec, Colorado, and Massachusetts. The wilt phase has been seen only in California.

This unusual *Ramularia* causes both a leaf disease and a stunt or wilt symptom of the entire plant resulting from systemic invasion of shoot and roots.

Sporulation of the fungus on the host may prove to be important in its local dissemination, and this should be considered in planning a control program for the disease.

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CERATOSTOMELLA ULMI TYPES IN RELATION TO DEVELOPMENT AND IDENTIFICATION OF PERITHECIA

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The discovery of perithecia of *Ceratostomella ulmi* (Schwarz) Buisman on field-infected elms in New Jersey and Colorado stimulated a study of several isolates of this fungus, from various parts of the United States, for their sexual capabilities and their morphological and cultural characters.

As early as 1932 Buisman (1) demonstrated that two races or types of *C. ulmi* were necessary for the production of perithecia in the laboratory. Later she found perithecia on European field-collected material (2). Following Buisman's work, Swingle (7) established the existence of two sexual strains in the United States, but he did not find perithecia in nature. Clinton and McCormick (3) obtained perithecia by crossing European with Connecticut isolates, but none were obtained by crossing isolates from the United States. None of these workers reported cultural or morphological differences between the sexual races. Walter (8) postulated an unlimited number of culturally different forms of *C. ulmi,* probably resulting from mutations, but did not pair various forms to determine their ability to produce perithecia. Fenner and Liming (4) reported on the dissimilar appearance of several isolates of *C. ulmi,* and on the similarities between some of these variants and other fungi isolated from elm wood.

In September 1947 the Dutch Elm Disease Identification Laboratory received bark and wood specimens from a bark beetle-infested elm in Morris County, New Jersey. The specimens were placed in moist chambers for 2 to 3 weeks at 55° F., and then were examined macroscopically for evidences of *C. ulmi* in the same manner that thousands of specimens had been examined in preceding years. Perithecia were found on both bark and wood. Specimens from Denver, Colorado, received in February 1948 and handled in the same manner, produced perithecia of *C. ulmi.* In April 1948 specimens from another New Jersey elm, only a few miles from the first reported tree, produced perithecia in the laboratory. Exuded ascospores from them produced typical colonies of *C. ulmi* on nutrient media and typical coremia on elm wood. Attention was not given to the cytogeny or physiology of perithecium production in *C. ulmi.*

The studies reported herein were limited largely to the recognition of isolates which alone did not produce perithecia under otherwise favorable conditions, but which produced perithecia when suitably paired on a favorable substratum. A few measurements and observations were made on cultural behavior.

MATERIALS AND TECHNIQUES

Fifty-three isolates of *C. ulmi* from diseased elm wood and from bark beetle-infested elm wood and bark were used in the tests. Most of the isolates were randomly selected to include material from various parts of the United States. However, a few were selected because they produced atypical growths on nutrient media, or perithecia on field-infected elm wood and bark. A few fungi with spore forms somewhat similar to those of *C. ulmi* were paired with *C. ulmi*. All isolates were obtained from diseased tissues or coremial heads, and none were of single-spore origin. The cultures were held on a potato infusion-sucrose agar medium.

Discs about $\frac{3}{8}$ in. thick were sawed from debarked elm stems $1\frac{1}{2}$ to $2\frac{1}{2}$ in. in diameter. The disc size was determined by the size of Petri dish used, allowing for space at the sides of the disc and above it. Two or three filter papers were placed in the bottom of the Petri dish, and about 20 ml. of tap water was added to each dish. The unit was then steam-sterilized at 15 lb. pressure for 1 hr. If water was lost during storage, sterile water was added at time of use to a depth of one-fourth the thickness of the disc. The filter paper reduced the free flow of water when the dish was tipped, and provided a suitable background for detecting fruiting structures on the sides of the disc and on the filter paper itself.

Potato infusion-sucrose agar and other synthetic media had already been found unsuitable for perithecium production. Of the woods other than elm that were tested, all were suitable for production of perithecia in some instances, but none were so consistently or so productively useful as elm wood.

Pairings were made by placing pea-size pieces of fungus-invaded medium from two isolates about 1 in. apart on an elm disc. Usually four dishes were made of each pairing. Although perithecia of *C. ulmi* will develop at a wide range of laboratory temperatures, the cultures were incubated at 55° F. Through a binocular, the disc surfaces and filter paper were examined for perithecia at the end of 18 days. Any heavy mycelial mat or thick yeast-like growth was scraped away in the search for hidden perithecia. The cultures were examined until perithecia were found, or at 3-week intervals up to 90 days. As the isolates showed evidence of belonging to a particular sexual type, additional crosses were made between members of a type and between types. Those isolates that showed evidences of mixed sexualism were paired with both monosexual types in many tests.

RESULTS OF PAIRINGS

Of the 53 isolates, 36 showed definite monosexual characteristics. Twenty-seven of them appeared to be of one type, herein called type A, because they consistently failed to produce perithecia when paired 384 times with each other. Another group of nine isolates, called type B, likewise did not produce perithecia when in-group paired 46 times. Of 240 pairings of type-A and type-B isolates, 217 produced perithecia on elm discs.

Twelve isolates showed some evidences of bisexualism. Eight isolates, classed as type Ab, produced perithecia 71 out of 72 times when paired with type-B isolates. However, some of them also produced occasional perithecia when paired with 9 of the 27 type-A isolates. Four isolates with strong type-B characteristics were classed as type aB because they produced perithecia in 101 of 106 pairings with type-A isolates, but also in a few instances with five of the nine type-B isolates.

Five isolates appeared to be definitely bisexual at the beginning of the test period or became so during the studies and were called type AB. Two of these originated from coremia produced on naturally infected elm bark and wood on which perithecia had been found. Early in the test period, both isolates apparently lost the ability to produce perithecia alone. Later they were paired with isolates of types A and B, and perithecia were produced only in pairings with type B. One type-AB isolate produced perithecia without pairing late in the test period, and had previously produced perithecia when paired with 18 isolates of type A, three of type Ab, one of type AB, three of type aB, and eight of type B. Another type-AB isolate produced perithecia when paired with 51 other isolates and failed only when paired with one type-B isolate. This isolate did not produce perithecia alone during the test period. The fifth type-AB isolate produced perithecia with 25 isolates of type A, eight of type Ab, two of type AB, one of type aB, and four of type B, but failed to produce perithecia when cultured alone.

The reasons for the performance of the five type-AB isolates, and to some extent that of the 12 isolates of types Ab and aB, were not determined. In type-Ab and type-aB isolates, one sex factor seemed stronger than the other. In two type-AB isolates both sex factors seemed strong, but not mutually compatible. In another type-AB isolate the two sex factors became mutually functional. In two type-AB isolates the B factor apparently was lost or became nonfunctional. It is possible that the B factor was eliminated from these isolates by chance during retransfers to maintain the cultures. The behavior of the mixed sexual isolates suggests the possibility that the type-A and type-B isolates may contain a currently nonfunctioning sex factor or are now monosexual as the result of the elimination of one sex factor by natural or artificial means.

The geographic origins of the isolates with respect to types as defined above are shown in table 1. Type A isolates were well distributed geographically and were the most frequent type in most of the Dutch elm disease areas. Type B and all but one of the type-aB isolates were from the Indianapolis, Indiana, area. Their restricted distribution supports the opinion that the Indianapolis infection developed independently of other infection areas. Type-AB isolates were not restricted geographically nor were they always associated with the type A most frequently isolated from the Dutch elm disease areas.

The percentage of the test isolates that were types aB and B may be

TABLE 1.—*Number of C. ulmi isolates of different sexual types from various geographic origins*

State of origin	Type A	Type Ab	Type AB	Type aB	Type B
Colorado	...	...	1	...	...
Connecticut	4	...	1	...	...
Indiana	1	...	...	3	9
Kentucky	1	1	...	...	...
Massachusetts	1	...	...	...	...
Missouri	1	...	...	...	...
New Jersey	4	2	1	...	...
New York	1	1	...	...	...
Ohio	7	2	1	1	...
Pennsylvania	2	...	...	...	...
Tennessee	3	...	1	...	...
Vermont	1	...	...	...	...
Virginia	...	2	...	...	...
West Virginia	1	...	...	...	...
Totals	27	8	5	4	9

higher than the natural occurrence of these types owing to the arbitrary inclusion of certain isolates because they were atypical. On the other hand, the percentage may be lower than average because atypical isolates may have been overlooked or considered as other fungi when randomized selections were made.

The perithecia in successfully paired cultures varied from one or two to several hundred on an elm disc. Most of the perithecia matured in about 3 weeks, but some did not develop until near the end of the 90-day test. A few negative cultures were held longer, but no perithecia developed. Frequently perithecia would develop on only one of three or four apparently similar discs.

The perithecia produced by artificial pairing were larger than those found on naturally infected elm material and also larger than those observed by earlier workers (Table 2).

The exudates of single perithecia produced by 100 pairings were trans-

TABLE 2.—*Measurements, in microns, of perithecia, asci, and ascospores of Ceratostomella ulmi*

Source	Perithecia			Asci		Ascospores	
	Diameter of base	Length of neck	Length of cilia	Diameter	Length	Width	Length
Paired A and B isolates	143–273	86–465	35–60	4.5–7.4	13.2–16.5	1.6	4.9
Infected field material	115–152	198–296	26–61	3.3–6.5	9.7–13.0	1.6–2.4	5.8–6.6
Buisman (1)	105–135	265–380	25–60	.....	.....	1.5	4.5–6.0
Swingle (7)	.....	.....	.....	3.1–6.8	8.8–15.0	.....	.....
Clinton and McCormick (3)	100–133	267–450	.....	.....	.....	1.5	5.0

Numbers in parentheses refer to literature citations.

ferred to a nutrient medium. Perithecia developed in only three Petri dishes, probably owing to the inadequacy of the substratum. Similar transfers to elm discs resulted in the production of perithecia in 211 of 272 (77.5 per cent) of the cultures. When similar mass transfers of ascospores from single perithecia were put on elm discs with a type-A or type-B isolate, perithecia developed in 100 per cent of the pairings. By a spore-dilution technique, 12 single-ascospore cultures were obtained. When these were paired on elm discs with type-A isolates, perithecia were produced in four instances. When paired with type-B isolates, perithecia were produced in one plate. The cytogenic factors involved in these results were not investigated.

On the discs containing types A and B, the coremia were more varied than in the usual isolates from diseased wood. On some discs, the coremia were very numerous but single, thin-stalked, and small-headed. In this respect they often resembled the coremia of other fungi. In some paired cultures the coremia were clumped and had stalks up to 123 μ in diameter. The stalks in some of these clumps were joined for three-fourths of their length from the base, and the spore heads coalesced into fan-shaped spore masses. Transfers of spores from such atypical coremia to sterile elm discs usually produced typical mycelial and spore forms of *C. ulmi*. On some discs there were areas of black tissue, probably the rudiments of coremia. Small sclerotiumlike bodies were more common in paired cultures than in most single isolates.

CULTURAL AND MORPHOLOGICAL DIFFERENCES BETWEEN TYPES A AND B

The varied appearance of the 53 stock cultures suggested the possibility of finding recognizable differences between the two types of *C. ulmi* involved in the production of perithecia. At the beginning of these studies, the 53 isolates, which were of varying ages, were transferred to elm discs in an attempt to reduce the chances of differences due to aging. Spores from coremial heads produced on the discs were transferred to the center of Petri dishes containing uniform amounts of a nutrient medium consisting of the infusion of 240 gm. of unpeeled white potatoes, 20 gm. of sucrose, 20 gm. of agar, and sufficient water to make 1 liter. The cultures were held at about 75° F. and observed at short intervals for growth rate of the colony, texture of the mycelial growth, amount of yeastlike spores, coloration, amount and nature of sectoring, and production of coremia. The occurrence, position, and size of spores were studied under the microscope. An isolate was classed as typical *C. ulmi* when its appearance agreed with the description given by Walter (8).

Of the 35 type-A and type-Ab isolates, 20 were classed as typical, and 15 varied only slightly from the recognized standard. Sectoring occurred more commonly in type-A than in type-B isolates. Of the 13 type-B and type-aB isolates, none produced typical growth. There was a pronounced tendency toward a greasy, yeastlike growth, indistinct zonation or none, and

an irregular colony outline not attributable to sectoring. Seven of the type-B isolates developed slow-growing colonies of compact white mycelium. Type-B isolates commonly produced more coremia than did type-A cultures. Three type-AB isolates produced colonies typical of *C. ulmi*, and two resembled type-B isolates. Although there was a greater frequency of irregularities in type-B than in type-A cultures on a nutrient medium, the macroscopic differences were not sufficiently consistent and distinct to permit an exclusive description of either type.

Sclerotia, as described by Clinton and McCormick (3), were absent or rare in unpaired isolates on nutrient medium, but were usually present in small numbers on elm discs, regardless of the type of isolate. Pairings of type-A and type-B isolates on nutrient medium regularly developed a few sclerotia, whereas pairings on elm discs developed numerous sclerotia.

The size of conidiospores produced by type-A and type-B isolates on potato-sucrose agar medium varied according to type. Variations in sizes of conidiospores have been reported by earlier workers. Representative measurements are in table 3.

TABLE 3.—*Sizes, in microns, of C. ulmi conidiospores from various sources*

Source	Width		Length	
	Range	Average	Range	Average
Type-A isolates	1.6–4.9	3.0	3.3–14.7	4.9
Type-B isolates	1.6–4.1	1.7	3.3–6.6	3.6
Clinton and McCormick (3)	2.0–3.5	2.4[a]	4.7–10.0	5.8[a]
May and Gravatt (5)	1.5–4.0		3.0–7.0	
Schwarz (6)	1.4–	2.0	2.5–7.0	4.0

[a] Size of attached spores. Spores tend to become wider and shorter when detached. Numbers in parentheses refer to literature citations.

The spore sizes obtained by Schwarz (6) agree closely with those of the type-B isolates. The spore sizes reported by Clinton and McCormick (3) are somewhat similar to those of the type-A isolates. The production of perithecia by pairing Connecticut and European isolates (3) also suggests that the isolates were of different types. The measurements of conidiospores of the type-B isolates tested, in most instances, were within the lower range of measurements of conidiospores of type-A isolates. Conidiospore size seems to be consistent and sufficiently different in the two types to permit identification of some isolates on the basis of this character.

RESULTS OF PAIRING C. ULMI WITH SIMILAR FUNGI FROM ELM

Other fungi somewhat like *C. ulmi* in one or more aspects have been isolated from elm wood. The growth rate, texture of mycelial mat, and zonation on potato-sucrose agar medium may resemble those of *C. ulmi*. The conidial, coremial, and perithecial fruiting structures may in some aspects be similar to those of *C. ulmi*. To test the possibility that one or more of

these fungi may be variants of or genetically related to *C. ulmi*, five of the somewhat similar fungi were paired with *C. ulmi* type-A and type-B isolates on elm discs. When a perithecium-producing fungus was involved, perithecia invariably were produced on the elm discs. Transfers of spores from such perithecia to a nutrient medium failed to produce growths recognizable as *C. ulmi*. In one instance, the presence of *C. ulmi* appeared to stimulate the production of perithecia by an unidentified fungus that had not previously produced perithecia in the laboratory.

SUMMARY

Perithecia of *Ceratostomella ulmi* were found for the first time (1947 and 1948) in the United States on naturally infected wood and bark collected from two elms in New Jersey and one elm in Colorado.

Fifty-three mass isolates of *C. ulmi* from elm were tested for ability to produce perithecia alone or paired on elm wood. They were classed as type A, Ab, AB, aB, or B.

Types A and B alone produced no perithecia, but when paired, perithecia were produced in 217 of 240 combinations.

Type Ab readily paired with types AB, aB, and B. Occasional perithecia were produced when Ab isolates were intra-paired and when they were paired with A isolates.

Type AB was inconsistent. Two isolates did not produce perithecia alone but did so when paired with either A or B. Three isolates alone in culture produced perithecia, but two of them lost the ability and pairing with B was necessary.

Type aB readily paired with types A, Ab, and AB, but did not ordinarily produce perithecia when intra-paired and when paired with type B.

The perithecia and asci resulting from paired isolates were larger than those found on naturally infected material.

Spore masses from single perithecia produced by pairing, when transferred to elm wood, produced perithecia in about three-fourths of the plates. When such transfers were paired with type-A or type-B material, perithecia were produced consistently.

Paired cultures usually failed to produce perithecia on potato-sucrose agar medium, probably because of inadequacy of the medium. The coremia produced in paired cultures on elm discs were more varied in size and shape than those produced by single isolates.

Although type-B isolates tended to be atypical, they could not be definitely distinguished macroscopically from type-A isolates. Type-B isolate conidiospores were largely within the lower limits of the range of sizes reported for type-A spores.

When five elm-inhabiting fungi, similar to *C. ulmi* in one or more as-

pects, were paired with *C. ulmi* on elm wood, there was no evidence that these fungi contributed to the production of *C. ulmi* perithecia.

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THE EFFECT OF SOIL TYPE ON THE DISPERSION OF SOIL FUMIGANTS

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INTRODUCTION

Practical field control of the sugar-beet nematode, *Heterodera schachtii* Schmidt, with soil fumigants containing a mixture of 1,3-dichloropropene and 1,2-dichloropropane[3] has been reported by Thorne and Jensen (12), who obtained satisfactory yields of sugar beets by treating heavily infested fields at the rate of 250 lb. per acre. In some tests yield increases of 11 to 15.6 tons of beets per acre were obtained. As a result of these investigations, soil fumigation with D-D mixtures has been recommended as an alternative method of controlling the sugar-beet nematode in the intermountain sugar-beet-growing area. Crop rotation, however, remains the principal method of control.

In California the results obtained with soil fumigants against sugar-beet nematode have been exceedingly variable in the field. In the majority of experimental tests and commercial demonstrations satisfactory increases in yield have not been obtained, primarily, it is believed, because of the failure of the soil fumigants to reduce the nematode population. Several explanations have been suggested to account for poor kills in the field: wrong time of treatment as related to possible biological differences, morphological differences such as might occur in the relative thickness of the cyst wall, higher populations, low soil moisture at the time soil fumigants are applied, and poor diffusion of the soil fumigants in certain California soils.

This paper presents the results of preliminary investigations on the efficacy of D-D fumigants in a number of California soils. A root-knot nematode, *Meloidogyne* sp., and the sugar-beet nematode have been used as test animals to determine the relative nematode kills obtained in the different soil types. Ethylene dibromide mixture was used in some experiments against root-knot and sugar-beet nematode.

LITERATURE REVIEW

Experimental investigations concerning the effective killing range of volatile chemicals used as nematocides have been reported by a number of

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[3] Shell D-D and Dowfume N. These materials are referred to as D-D mixture in the remainder of the text.

workers. In general these investigations have had as their objective the development of experimental techniques to be used in comparing the nematocidal efficacy of various chemicals. Newton et al. (8) devised a method that can be used to determine the effective killing range of fumigants against *Ditylenchus dipsaci* (Kuhn), the bulb or stem nematode. They placed samples of the nematode inoculum 6 in. below the surface of the soil and determined, on the basis of mortality, the effective lateral killing range of the chemical. Chitwood (1) modified these techniques in order to obtain vertical as well as lateral killing range.

Christie (3) reported the results of tests in which the lateral killing range at a 6-in. depth was determined for several chemicals in Berwyn loam. In similar tests (4) using sand as a substrate in a gas-tight box $4 \times 4 \times 48$ in., tightly closed during the fumigation period of 7 days, the lateral killing range of 2 ml. of each fumigant under consideration was determined.

McClellan et al. (6) have reported the effect of soil temperature and soil moisture on the efficacy of soil fumigants. Their results indicate that the fumigants used were most effective in wet soils and were retained longest in wet, low-temperature soils. Schmidt (9) compared the dispersal of D-D mixture and chloropicrin in dry, moderately wet, and wet soil. He concluded that vapor movement was most rapid in moderately wet soil and least rapid in dry and wet soil. The effect of soil moisture upon the lateral and vertical movement of D-D mixture and chloropicrin was also studied.

There appears to be little or no literature comparing the nematocidal properties of soil fumigants in different soil types. However, several workers have investigated the physical properties of soils in relation to the diffusion of fumigants. Such factors as compaction, pore space, moisture content, temperature, and soil type have all been shown to influence the dispersal of gases in soil. The role of adsorption has been investigated by Stark (10). He found that the amount of chloropicrin adsorbed by the soil is largely dependent upon the colloidal clay fraction. Colloidal clay adsorbed nearly 10 per cent of its dry weight as compared to 0.18 per cent for sand, 0.8 per cent for silt, and 3.2 per cent for clay. He also found that the addition of ground charcoal to the soil completely inhibited the toxic action of a given dosage of chloropicrin. Hagan (5), studying the movement of carbon disulfide vapor through the soil, concluded that moisture exerted a greater influence upon soil permeability than such factors as porosity and soil compaction. He found that permeability varied with soil texture, but concluded that the differences observed were probably smaller than would be expected in the field. Adsorption as a factor in permeability was not studied by Hagan (5) since he was working with constant flows of the gas through different types of soils under controlled conditions.

MATERIALS AND METHODS

Galvanized metal cans 20 in. wide and 26 in. high with a capacity of 32 gal. were found to be satisfactory containers for experimental soils. This volume of soil is adequate for the study of killing ranges 8 in. laterally from the point of injection and to a depth of at least 18 in. below the surface. The cans facilitate the handling and moving of large quanties of soil and make excellent containers for storage; with tight-fitting lids, the soil moisture can be maintained at a nearly constant level.

The soils used in these experiments were surface soils collected from cultivated fields and screened through a $\frac{1}{4}$-in. screen in the laboratory. A 32-gal. can was filled with each type of soil and kept tightly covered when fumigation experiments were not in progress, to minimize loss of water. Soils were not allowed to become air-dry. To maintain them in a condition approximating that of a seedbed in the field, the soil was spread in a thin layer on a concrete floor, the desired volume of water was sprinkled on, and the soil was thoroughly mixed and replaced in the can. Several days elapsed before the soils were used in fumigation treatments.

Preparation of nematode inoculum. It is desirable, in tests involving nematode kills, to have available a uniform source of the nematode inoculum. Several investigators have used the ground-up roots of plants heavily infested with root-knot nematode as a test sample to determine the killing range and toxicity of soil fumigants. Samples of this inoculum are usually placed in a cheesecloth bag and exposed to the action of the nematocide. In some instances soil has been mixed with the ground-up root material. In this type of inoculum it is probable that the kills obtained are a measure of the fumigant's toxicity against larvae in the soil and against eggs. It is also probable that the inoculum contains eggs in several stages of embryonation. It seems likely that under these conditions the kills obtained might vary according to the percentage of eggs and larvae in the inoculum. In order to avoid this possibility the inoculums used in these experiments were prepared so that the action of the nematocide was tested against either second-stage larvae of root-knot nematode or the eggs of the sugar-beet nematode. It is believed that in the field these are the stages that must be killed in order to obtain satisfactory control.

The root-knot nematode inoculum was prepared by burying the washed roots of heavily infested tomato plants in a quantity of Yolo fine sandy loam for 10 days. The roots were then carefully removed and the soil was screened and thoroughly mixed. This inoculum was stored in tightly closed containers for several days before use in fumigation tests. Inoculum prepared in this manner can be stored for several weeks if kept cool. This procedure yields an inoculum with practically all root-knot nematodes in the second larval stage. Any eggs dislodged from the tomato roots probably hatch during storage of the inoculum.

Sugar-beet nematode inoculum consisted of soil from a field of beets

heavily infested with the nematode. This soil was screened, mixed, and then stored in a tight container. The sugar-beet nematode has a biology somewhat different from that of the root-knot nematode, and in order to obtain reasonable control in the field it is probably necessary that the fumigant have an ovicidal action. In the field at the time fumigants are applied the bulk of the sugar-beet nematode population consists of brown cysts, i.e., eggs enclosed in the dead bodies of the females. In the inoculum used in these tests, more than 90 per cent of the nematodes were in the egg stage. Since sugar-beet nematode inoculum can be stored for long periods in the laboratory, inoculum from the same source could be used in all of the tests involving this nematode.

Fumigation of soil. Evidence of lateral and vertical movement of the fumigant was obtained by determining the kill of nematodes in 100-ml. samples of inoculum in cheesecloth bags, placed in the soil prior to fumigation, at various distances from the point of injection. Fumigants were injected 6 in. below the soil surface and 10 in. from the sides of the container. Duplicate samples were placed 2, 4, 6, and 8 in. from the injection center and 3, 6, 12, and 18 in. below the surface of the soil. In each test, 32 samples were exposed to the action of the fumigant.

After remaining 7 days in the fumigated soil, which was uncovered and undisturbed during this time, the root-knot nematode samples were removed and mixed with enough nematode-free soil to fill a 4-in. pot. One tomato seedling was then transplanted into each pot. After 4 weeks the roots of these seedlings were washed and the number of galls on the roots was used as an indication of effectiveness of the fumigant. A similar procedure was used with sugar-beet nematode samples, except that sugar-beet seeds were planted in the mixture of nematode-free soil and inoculum. Counts were made of the number of nematodes on these seedlings. With both root-knot and sugar-beet nematode samples, roots were examined only until 100 nematodes had been recorded. We believe that counts of this magnitude indicated that the fumigant had little or no effect upon the nematodes in the sample. In each instance this assumption has been checked by examination of a number of untreated samples.

Fumigation tests were carried out within the range of 18° to 22° C., averaging about 20° C. during the 7-day exposure period. An attempt was made to fumigate when the moisture content was about 75 per cent of the moisture equivalent of the particular soil. This approximates conditions thought to be favorable for the application of nematocides in the field.

Soil moisture was determined on an oven-dry basis. With the exception of the samples of Egbert organic loam (peat), the soil samples were heated for 24 hr. at 110° C. Peat samples were heated for 24 hr. at 70° C. Moisture equivalents[4] were determined by the Briggs and McLane method as modified by Viehmeyer et al. (13).

[4] Moisture equivalents were kindly determined by Dr. E. F. Feichtmeir of the Shell Agricultural Laboratory, Salida, California.

RESULTS

Effect of soil type on root-knot kills. Field observations made during the 1948 growing season indicated that D-D mixture failed to control sugar-beet nematode in clay and peat-type soils at dosages up to 400 lb. per acre. Similar situations were observed during the 1946 season. The possibility was suggested that only limited diffusion of the soil fumigant occurred in these soils. Field soils with moisture equivalents ranging from 7.0 to 150 (Table 1) were collected and tested in the laboratory. The effective diffusion range (Table 2) of 2.15 ml. of D-D mixture is indicated by the survival of root-knot nematode larvae in samples placed various distances from the injection point of the fumigant in the different soil types.

TABLE 1.—*Soil types, moisture equivalents, soil moisture, and temperature at the time of treatments to determine the effect of soil type on diffusion of D-D mixture*

Soil type	Moisture equivalent	Moisture (per cent)	Temperature (°C.)
Fresno sandy loam	7.0	5.1	22
Yolo fine sandy loam	10.4	9.2	19
Yolo silt loam	18.6	15.5	20
Yolo clay loam	30.1	23.9	20
Bowers clay	31.7	23.2	20
Egbert organic loam	150.0 (approx.)	112.5	20

The data in table 2 show that root-knot nematode larvae located a short

TABLE 2.—*Lateral and vertical diffusion of 2.15 ml. of D-D mixture in six soil types as indicated by the numbers of root-knot nematode larvae surviving in 100-ml. samples of inoculum located various distances from the point of injection, indicated by X*

Depth, inches	Lateral distance—inches								Lateral distance—inches							
	8	6	4	2	2	4	6	8	8	6	4	2	2	4	6	8
	Fresno sandy loam								Yolo fine sandy loam							
3	0	0	0	0	0	0	4	14	25	17	0	0	0	0	4	42
6	1	0	0	0	X 0	0	1	0	0	0	0	0	X 0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total					20								88			
	Yolo silt loam								Yolo clay loam							
3	100	90	84	0	4	52	100	86	100	90	10	31	0	32	33	67
6	49	5	0	0	X 0	0	20	23	37	21	0	0	X 0	0	4	59
12	21	47	3	0	1	11	19	9	18	0	4	3	0	0	3	85
18	94	93	100	86	100	100	100	100	100	43	100	62	46	100	57	53
Total					1507								1155			
	Bowers clay								Egbert organic loam							
3	100	100	43	26	65	100	100	100	100	100	22	0	0	18	100	100
6	100	100	0	0	X 1	100	65	100	100	33	0	0	X 0	0	14	97
12	100	100	100	5	.....	100	100	82	100	100	100	100	100	67	100	96
18	100	100	100	100	28	100	100	100	100	100	100	100	100	100	100	100
Total					2417								2247			

distance from the injection point were able to survive treatment in soils containing organic material or clay. This would indicate that failure to control sugar-beet nematode in the field might be associated with dispersal of the fumigant in the soil. It is not possible from this type of investigation to determine the factors that limit the diffusion of gases in the heavier types of soil. However, it is probable that the factors indicated by other workers to have an influence on the diffusion of gases in soil are all operative in the present experiments. There is a correlation between either the moisture equivalent or the percentage moisture, or both, and the diffusion of the nematocide as measured by the nematode kills obtained in the various soil types. The efficacy of the fumigant in the different soil types is indicated by the total number of larvae surviving the treatment in each soil (Table 2).

TABLE 3.—*Lateral and vertical diffusion of 2.15 ml. of D-D mixture in four soil types as indicated by the number of sugar-beet nematodes surviving in 100-ml. samples of inoculum located various distances from the point of injection, indicated by* X; *temperature 21° C.*

Depth, inches	Lateral distance—inches								Lateral distance—inches							
	8	6	4	2		2	4	6	8	8	6	4	2		2	4	6	8
	Fresno sandy loam (5.1)[a]									Yolo fine sandy loam (10.1)								
3	0	0	0	0		0	0	0	0	0	0	0	0		0	0	2	23
6	0	0	0	0	X	0	0	0	0	0	1	0	0	X	0	0	0	0
12	0	0	0	0		0	0	0	0	0	0	0	0		0	0	0	0
18	0	0	0	0		0	0	0	0	3	11	8	1		2	7	1	3
	Bowers clay (25.0)									Egbert organic loam (108.0)								
3	43	7	24	0		0	0	100	52	100	100	43	10		6	26	20	100
6	6	0	0	0	X	0	0	0	11	89	38	21	0	X	0	2	100	100
12	53	0	0	1		1	0	65	1	100	100	41	18		68	100	42	63
18	38	100	66	100		100	92	26	49	21	100	41	100		100	10	15	100

[a] The number in parentheses indicates the moisture percentage of the soil at time of treatment.

It appears from these data that D-D mixture at a given dosage is much less effective in killing root-knot nematode larvae in clay-type soils and soils high in organic matter, because of limited dispersal of the fumigant. In these tests the fumigant was least effective in killing nematodes in the 3-in. and 18-in. levels. It was most effective at the 6-in. and 12-in. depths.

Effect of soil type on sugar-beet nematode kill. The effect of soil type upon the kill of sugar-beet nematode was studied, using the same methods and soil as were employed in the root-knot nematode experiment (data in table 3). The kills obtained in the sandy soils in this test were better than had been anticipated. It has been the general assumption among nematologists that the sugar-beet nematode is more resistant to soil fumigants than is the root-knot nematode, because of the former's resistant cyst stage in its life history—the stage that is present in the field at the time soil fumigants are applied. Thorne and Jensen (12) suggest that the sugar-beet nematode presents one of the most difficult problems in soil fumiga-

tion because of the brown-cyst stage, in which the eggs are very effectively protected, and that the killing range of chemicals against sugar-beet nematode is less than half that at which most other plant-parasitic nematodes are killed; consequently, a heavier dosage will probably be required for successful control.

The data in table 3 indicate that the sugar-beet nematode can be killed in sandy soil with D-D mixture at about the same dosage that is effective against the root-knot nematode, and that killing range of the chemical is about the same against both species. A series of experiments was set up to test the susceptibility of the two species to the fumigant. Two 100-ml. samples of inoculum, one of root-knot nematode and one of sugar-beet nematode, were placed in each of twelve 2-qt. jars filled with Fresno fine sandy loam; the fumigant was added to the soil surface and the jars sealed for 24 hr. Twelve dosage rates of the fumigant ranging from 0.025 to 2.00 ml. were used in the experiment. All of the root-knot and sugar-beet nematodes were killed by the lowest dosage (0.025 ml. per 2-qt. jar) used in the test.

The experiment was repeated using lower dosages of D-D mixture. Each dosage rate was run in duplicate. The results (table 4) indicate

TABLE 4.—*Relative susceptibility of root-knot and sugar-beet nematodes to D-D mixture in closed containers (temperature 21.5° C.), as indicated by the number of nematodes surviving at each dosage rate*

Amount of fumigant, in ml., per 2-qt. jar	Sugar-beet nematode		Root-knot nematode	
	A	B	A	B
0.005	100	68	100	100
0.01	100	10[a]	100	100
0.02	0	13	0	0
0.03	0	0	0	0

[a] All seedlings died; soil washed to recover these larvae.

that eggs of the sugar-beet nematode and larvae of the root-knot nematode are about equally susceptible to the toxic action of D-D mixture. This is in agreement with the data in table 3 and indicates that this fumigant has a very high ovicidal action against sugar-beet nematode eggs as well as being an excellent larvicide.

The efficacy of D-D mixture in Yolo loam and Yolo clay. These soil types were not obtained in time for inclusion in the first experiments, but were tested separately using the technique of including both root-knot and sugar-beet nematode in the same fumigation test (Table 5).

The results (Table 5) again indicate that D-D mixture is about equally effective against both root-knot and sugar-beet nematode. Yolo clay and Yolo loam as used in this experiment tend to restrict the movement of the fumigant so that toxic concentrations were not attained within 6 in. laterally from the point of injection. Toxic concentrations occurred at least 6 in. vertically from the point of injection, indicating that the movement

of the fumigant tends to be downward from the point of injection in these soils. In these and similar tests we believe that root-knot nematode kills are a more accurate measure of the movement of the nematocide than are sugar-beet nematode kills, because of the difficulty of making counts and the possibility of dislodging females of the sugar-beet nematode. However, it is evident from the results obtained in this series of experiments that it is worth while to consider the action of fumigants from the kills obtained upon the various stages of parasitic nematodes that are encountered in the soil.

Susceptibility of root-knot and sugar-beet nematodes to ethylene dibromide mixture. Thorne and Jensen (12) reported that treatment of sugar-beet nematode infested soil with ethylene dibromide mixture was not so effective as were treatments with D-D mixture. The authors have ob-

TABLE 5.—*Lateral and vertical diffusion of D-D mixture in Yolo loam and Yolo clay*[a] *as indicated by the number of root-knot and sugar-beet nematodes surviving in 100-ml. samples of inoculum placed various distances from the point of injection, indicated by X*

Depth, inches	Number of nematodes surviving															
	Root-knot					Sugar-beet				Root-knot					Sugar-beet			
	Lateral distance—inches															
	8	6	4	2		2	4	6	8	8	6	4	2		2	4	6	8
	Yolo loam									Yolo clay								
3	80	100	78	6		3	7	85	61	100	100	63	25		28	64	46	63
6	36	11	0	0	X	0	0	0	8	100	92	0	0	X	0	8	0	34
12	10	8	0	0		0	0	0	13	76	78	0	0		0	3	15	9
18	100	100	100	100		71	10	67	45	100	100	100	100		71	29	100	27

[a] Moisture equivalent: loam, 18.6; clay, 27.8. Percentage moisture at time of treatment: loam, 11.8; clay, 16.0.

served fields in the Salinas Valley, California, in which ethylene dibromide mixtures failed to control the sugar-beet nematode in sandy soil. Ethylene dibromide mixture was injected into Yolo fine sandy loam at several dosage rates, and both root-knot nematode and sugar-beet nematodes were included in each test. The data (Table 6) indicate that ethylene dibromide mixture is not an effective nematocide for use against the sugar-beet nematode. There was no appreciable reduction in the number of sugar-beet nematodes in samples located 2 in. from the point of injection of the nematocide. It is evident by the kills obtained against the root-knot nematode that the fumigant was able to diffuse readily in the sandy soil. In these tests 0.86 ml. of ethylene dibromide mixture appears to be of about the same effectiveness against the root-knot nematode as 2.15 ml. of D-D mixture. It appears probable that the failure of ethylene dibromide mixtures to kill the sugar-beet nematode was due to the fact that most of the sugar-beet nematodes were in the egg stage. This would indicate that this fumigant is not toxic to the eggs of sugar-beet nematode. If this is the

case, it would be logical to expect that ethylene dibromide mixture would not be effective in the field against sugar-beet nematode. Ethylene dibromide mixture appears to be very toxic to larvae of the root-knot nematode, but whether it is toxic to the eggs of this nematode remains to be determined. In any event, good kills can usually be obtained with ethylene dibromide against root-knot nematode in sandy type soils because the larvae are susceptible.

DISCUSSION

Results obtained in laboratory tests are usually not a reliable indication of the effect that a fumigant might have in the field. For example, it is well known that it is not necessary to kill all of the plant-parasitic nema-

TABLE 6.—*Relative susceptibility of sugar-beet nematode and root-knot nematode to various dosages of 40 per cent by weight ethylene dibromide mixture applied in Yolo fine sandy loam,[a] as indicated by the number of nematodes remaining alive in 100-ml. samples of inoculum; injection point of fumigant indicated by* X

Depth, inches	Number of nematodes surviving															
	Root-knot					Sugar-beet				Root-knot				Sugar-beet		
	Lateral distance—inches															
	8	6	4	2		2	4	6	8	8	6	4	2	2	4	6	8
	Dosage 0.43 ml.									Dosage 0.86 ml.							
3	100	44	35	66		100	100	41	100	6	26	2	0	36	39	100	100
6	0	2	0	0	X	100	100	100	100	0	0	0	0	X 35	48	39	72
12	0	3	0	0		73	89	100	67	0	0	0	0	100	34	78	80
18	0	1	0	0		10	54	100	100	0	0	0	0	100	100	100	91
	Dosage 1.07 ml.									Dosage 2.15 ml.							
3	0	0	0	0		100	100	100	100	0	0	0	0	100	100	100	100
6	0	0	0	0	X	100	100	100	100	0	0	0	0	X 100	100	100	100
12	0	0	0	0		100	100	48	100	0	0	0	0	100	100	100	100
18	0	0	0	0		100	46	100	51	0	0	0	0	59	100	100	12

[a] Temperature 21.5° C. Soil moisture 5 per cent, moisture equivalent 10.4 per cent.

todes in the soil in order to obtain significant increases in yield. It is indeed an unusual occurrence if 90 per cent of the nematodes are killed by the ordinary dosage used in the field. There are almost no data available to indicate the magnitude of the reduction of root-knot nematode population that is necessary in order to grow a profitable crop. Newhall (7) has indicated that D-D mixture is a satisfactory nematocide against root-knot nematode if used at 500–700 lb. per acre. In California the recommended dosage against this nematode is 200 lb. per acre. This amount of the fumigant usually reduces the population to the extent that very satisfactory increases in yield are obtained. No attempt is made to obtain complete control of the nematode. Chitwood and Feldmesser (2), working with the golden nematode of potato, *Heterodera rostochiensis* Wollenweber, have indicated that although a treatment will cause 90 per cent mortality, the nematode population will be only slightly reduced at

harvest and light crop injury will result. Treatments resulting in 99 per cent plus kill are said to permit the growing of two potato crops without nematode damage. There is no doubt that in many instances it is necessary to kill only enough nematodes so that the plants are well established before they are heavily attacked. However, in the case of carrots and similar root crops, the control must be very good or crop loss will result, since it is the practice to cull root-crops that show obvious evidences of nematode injury.

It should be pointed out that the results recorded here represent only the effect of the fumigant from a single injection point. In the field such fumigants as D-D mixture and ethylene dibromide mixture are applied in continuous lines or injected at points located about 12 in. apart. From the combined action of the fumigant on the soil area between the injection points or the lines of injection, lethal concentrations might result at distances where no kill is obtained by a single injection.

The fact that root-knot nematode larvae and eggs of the sugar-beet nematode are about equally susceptible to D-D mixture suggests that this material is a very effective ovicide. Stark and Lear (11) have shown that D-D mixture is an effective fumigant when used to treat unrotted root-knot nematode galls in which many eggs were present. Their results may also indicate that D-D mixture is effective against both eggs and larvae of this species.

Ethylene dibromide mixture is an effective larvicide against root-knot nematode larvae, but appears to have little or no effect against the eggs of the sugar-beet nematode. This suggests that in field control ethylene dibromide mixtures may not be highly effective if nematode eggs are present in the soil or in root debris of a previous crop. On the basis of the results obtained in these laboratory tests, the authors believe that ethylene dibromide should not be applied in the field for the control of sugar-beet nematode.

SUMMARY

D-D mixture applied in cans of soil at the dosage rate of 2.15 ml. at a single injection point effectively killed root-knot nematode larvae and eggs of the sugar-beet nematode at least 8 in. laterally and 12 in. below the point of injection in sandy-loam soils. In these and other soil types the fumigant was least effective in samples located 3 in. below the surface. The lateral and downward vertical movement of the fumigant was reduced in soils having a relatively high moisture equivalent.

D-D mixture was equally effective against root-knot nematode larvae and eggs of the sugar-beet nematode. Ethylene dibromide mixture was found to be an effective fumigant against root-knot nematode larvae, but failed to kill sugar-beet nematode eggs.

It is suggested that a partial explanation for the noneffective control of sugar-beet nematode in California by D-D mixture is the fumigant's

failure to diffuse through soils containing clay and those high in organic material. A given dosage of D-D mixture appears to be about 100 times more effective in Fresno sandy loam than in Bowers clay or Egbert organic loam, because of its greater dispersal in the light soil. Soils tested which were about intermediate in moisture equivalent between Fresno sandy loam and Yolo clay were about intermediate in their permeability to the fumigant, as indicated by the nematode kills obtained.

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PHYTOPATHOLOGICAL NOTES

A Modification of Moore's Method of Inoculating Wheat and Barley with Loose Smut.[1] W. J. CHEREWICK AND W. POPP. Extensive studies of physiologic specialization in the loose smuts of wheat and barley and the testing of varieties and selections of these two cereals for their reactions to loose smut require a rapid and effective method of inoculation. Various methods have been used but most of them, even though effective, are too slow for large-scale tests.

In 1936, Moore[2] described a partial vacuum method of inoculation, in which a suspension of spores was introduced into the florets of heads placed in a glass chamber from which the air could be evacuated. This method enabled one person to inoculate as many as 30 heads per hour. It was quite effective on wheat, producing from 90 to 100 per cent infection on susceptible hosts, but was less effective on barley.

Shands and Schaller[3] thoroughly reviewed the various methods of loose smut inoculation and retested on barley the three methods most commonly used. They modified Moore's vacuum method by using a larger Dewar glass cylinder so that "2 or 3 and sometimes 4 heads could be inoculated at the same time." None of the methods tested proved very effective with barley. They[3] also injected dry spores into barley florets by means of a hypodermic needle to which was attached a small rubber bulb containing the spores. The latter method proved to be the most effective and the one most easily manipulated.

Of the various methods of inoculating wheat and barley with loose smut tested in this laboratory, Moore's partial vacuum method, with a slight modification, has proved to be the quickest; it is also very effective. The modification consisted of introducing a valve into the pump so each consecutive stroke of the pump exhausted more air and thus created a higher vacuum. A valve from a small atomizer was used. A $\frac{1}{4}$-in. copper pipe "T" was inserted into the suction tube just above the inoculation chamber (Dewar glass cylinder), and a piece of rubber tube was fitted on the free end of the "T". The tube was clamped with a pinchcock as described by Oort.[4]

With the pinchcock closed, a gentle stroke of the pump withdraws the

[1] Contribution No. 1000 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Moore, M. B. A partial vacuum method for the inoculation of wheat and barley with loose smut. Phytopath. **26**: 397–400. 1936.

[3] Shands, H. L., and C. W. Schaller. Response of spring barley varieties to floral loose smut inoculations. Phytopath. **36**: 534–548. 1946.

[4] Oort, A. J. P. Inoculation experiments with loose smuts of wheat and barley (*Ustilago tritici* and *U. nuda*). Phytopath. **29**: 717–728. 1939.

air from the glass cylinder, which, in turn, becomes filled with spore suspension. As soon as the enclosed head is covered with the suspension the tube between the cylinder and the suspension-container is pinched by hand and then a few rapid strokes of the pump create a vacuum of about 25 in. of mercury in the glass cylinder. This exhausts the air from the florets of the enclosed head and replaces it with the spore suspension. The pinchcock is then opened and the sudden rush of air into the cylinder drives still more of the spore suspension into the florets.

Using this modification and with a very soft rubber stopper that holds the head in the glass cylinder, it was possible to obtain, in susceptible varieties of barley, over 90 per cent infection. When two persons worked together, one manipulating the heads into the glass cylinder and the other tagging the inoculated plants and operating the pump, it was possible to inoculate as many as 100 heads per hour.

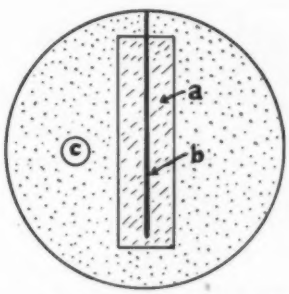

FIG. 1. Split rubber stopper for loose smut inoculation: (a) sponge-rubber insert; (b) cut through the stopper and sponge insert; (c) copper tube for intake and drain of spore suspension.

Since 1941 soft rubber stoppers have not been available and the ordinary black or gray stoppers have proved to be too hard to hold barley heads in the glass cylinder without excessive leakage of the air or injury to the necks of the inoculated heads. Difficulties encountered with the ordinary rubber stoppers led to another modification of Moore's method.

A larger Dewar glass cylinder with a diameter of 5 cm. was used as an inoculation chamber. A No. 11 rubber stopper was carefully shaped to fit closely the opening of the cylinder. In the middle of the stopper a rectangular hole 10×40 mm. was cut and the walls of the hole were trimmed to make them smooth and parallel. A piece of sponge rubber 12×42 mm. was forced into the hole of the stopper and fixed to the latter with a black paste cement.[5] The inlaid sponge rubber and the stopper were then cut through, as illustrated in (b) of figure 1, care being taken to make a straight, smooth cut. At one side of the sponge-rubber insert, a round hole was bored through the stopper and a short piece of tightly fitting copper tube was forced into the hole for the connection of the spore-suspension suction tube.

[5] The adhesive sold as "Miracle" cement has been most satisfactory.

The oversize sponge-rubber insert keeps the split cork partially open and so facilitates inserting the heads of grain to be inoculated. Furthermore, when the cork is closed over a number of peduncles and forced into the glass cylinder, the sponge rubber makes a perfect seal without the slightest injury to the stems.

With the apparatus just described 5 to 10 heads of barley, or 10 to 15 heads of wheat, can be inoculated at one time. Six to 10 rapid strokes with a pump make sufficient vacuum to insure effective inoculation of all flowers in the heads even if, as with barley, they are partially enclosed in the sheaths. Two persons working with the modified apparatus can inoculate from 400 to 600 heads per hour, depending on the efficiency of the helper in tagging the inoculated plants.—Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba.

Ascochyta Blight of Okra in Western North Carolina. D. E. ELLIS. Severe epiphytotics of Ascochyta blight of okra (*Hibiscus esculentus* L.) occurred in trial plots, home gardens, and small commercial plantings near Hendersonville, North Carolina, in 1948 and again in 1949. The disease appeared to be a limiting factor in the production of okra in that area, where recent attempts have been made to grow it commercially for freezing. The blight was first noted in July in the form of a few scattered leaf spots, but rapidly increased in severity, affecting blossoms, pods, stems, petioles, and leaf blades. It was most severe in September and continued to develop until frost. By mid-September in both years defoliation ranged from 50 to 90 per cent, and flower and pod infection was so severe that yields were practically nil. The disease was equally severe on the three varieties grown; Clemson Spineless, Louisiana Green Velvet, and Perkins' Spineless.

The fungus found fruiting abundantly on all affected parts appears to conform to the description of *Ascochyta abelmoschi* Harter.[1] In addition to pod and stem symptoms described by Harter, lesions have been noted also on leaves and flowers. Typical spots on the leaf blades are irregular in outline, with purple to black margins and gray centers bearing pycnidia arranged in a roughly concentric manner (Fig. 1, C). The necrotic centers frequently shatter out leaving an irregular shot-hole. Petiole lesions are similar to those on stems. Lesions also commonly occur along the leaf veins. These leaf symptoms have been reproduced by artificially inoculating young plants grown under glass. Unopened buds often are infected (Fig. 1, A) and the flower is destroyed before pods are formed. The fungus frequently spreads from affected blossoms to stems with which they come in contact (Fig. 1, D, E). Infection on one side of the receptacle often results in the development of curved pods. Vascular discoloration similar to that noted in pods by Harter was also found associated with stem and petiole lesions.

[1] Harter, L. L. A hitherto-unreported disease of okra. Jour. Agr. Res. [U.S.] 14: 207–212. 1918.

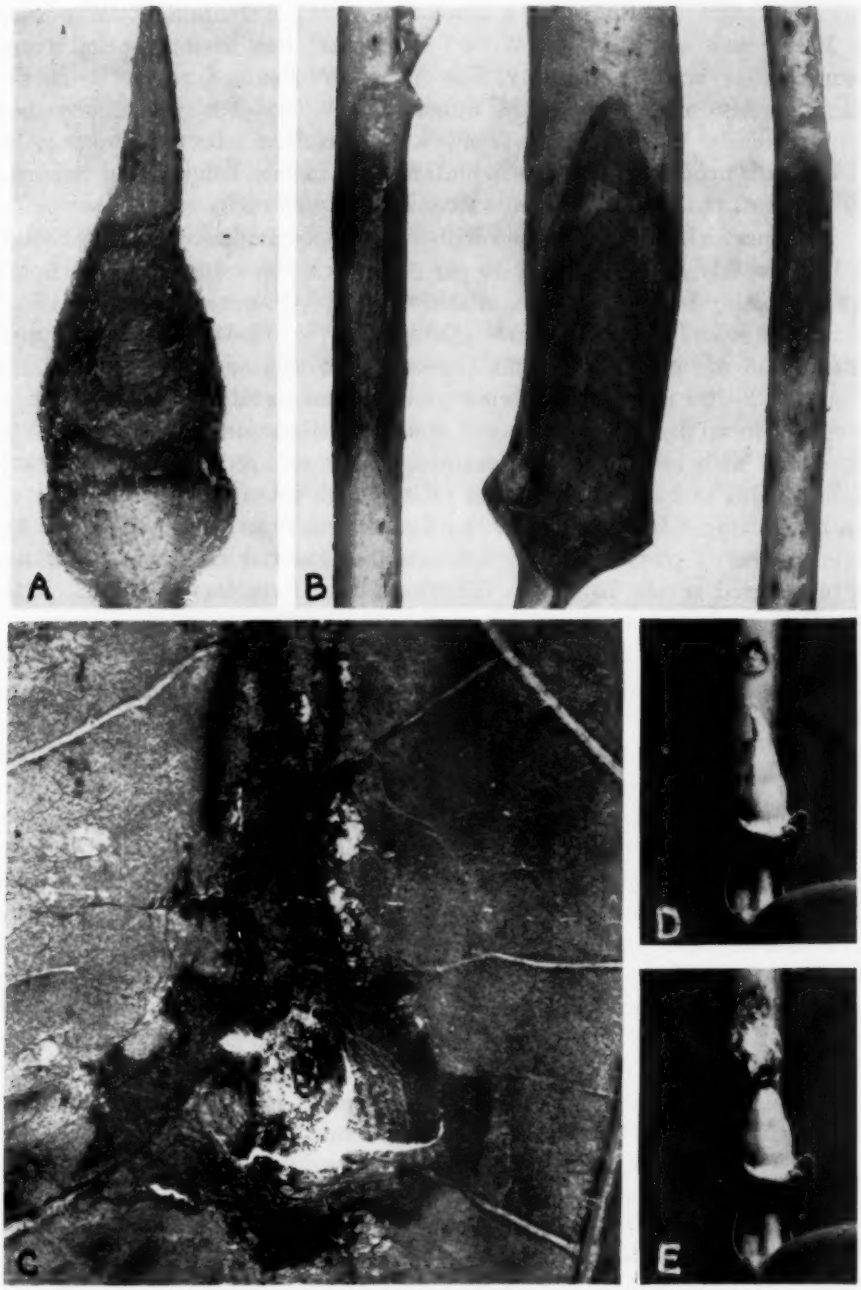

FIG. 1. Symptoms of Ascochyta blight of okra. A. Lesion on unopened flower bud. B. Lesions on stems and pod. C. Lesion on portion of leaf-blade. D. Stem lesion resulting from infection from an affected blossom, as shown in E.

Since Harter described *A. abelmoschi* and showed that it caused okra pod blight in 1918 only a few records of its occurrence have been pub-

lished. It was mentioned as a disease of okra in Georgia by Woodroof in 1927,[2] and according to Weiss' check list[3] has been reported from Georgia, Maryland, New Jersey, New York, and Pennsylvania. Its occurrence has also been recorded in Bulgaria[4] and Ceylon.[5] The disease has not previously been reported from North Carolina. Its development in such severe proportions in the mountain area in late August and September suggests that the organism is favored by relatively cool weather.

In a preliminary spray test[6] with the variety Louisiana Green Velvet in 1949, weekly applications of 49 per cent tetrachlorobenzoquinone (Spergon, wettable) 4–100, Bordeaux mixture 8–8–100, 76 per cent ferbam (Fermate) 2–100, 65 per cent zineb (Dithane Z-78) 1½–100, tribasic copper sulfate (53 per cent Cu) 4–100, lime-sulfur 2–100, or 76 per cent ziram (Zerlate) 2–100 resulted in higher yields of marketable green pods than were produced by the nonsprayed control. High yields were positively correlated with low defoliation ratings. Plots sprayed with ferbam gave higher yields and lower defoliation ratings than those sprayed with any of the other fungicides. However, the disease was relatively severe even on ferbam-sprayed plots and control was not considered satisfactory. Lime-sulfur caused severe injury to foliage.—Plant Pathology Section, North Carolina Agricultural Experiment Station, Raleigh, North Carolina.

A Tumor-Inducing Bacterium Isolated from Euonymous Galls. ARMIN C. BRAUN. During the course of an investigation designed to characterize the agent responsible for the initiation of large tumorous overgrowths present in a heavily infested Euonymous (*Euonymous fortunei radicans*) hedge, a bacterium was consistently isolated that was capable of inciting the formation of typical crown-gall tumors on tomato, sunflower, *Kalanchoe*, and sugar-beet plants. Little or no pathogenic reaction was produced by this organism, however, on the Paris daisy. The *Euonymous* isolates differed from typical strains of *Agrobacterium tumefaciens* in that they grew sparsely and without a pellicle on Difco nutrient dextrose broth, and lacked entirely the ability to initiate growth in a mineral salts medium containing dextrose as the carbon source. Both media supported abundant growth of four different stock strains of the crown-gall bacterium. The *Euonymous* isolate possessed the morphological, tinctorial, and disease-producing properties of *A. tumefaciens*, and it therefore appeared that this organism was a biochemically deficient mutant of the crown-gall bacterium. Since such naturally occurring variants have not yet been de-

[2] Woodroof, J. G. Okra. Ga. Exp. Sta. Bul. 145: 164–185. 1927.

[3] Weiss, Freeman. Check list revision. U. S. Dept Agr., Pl. Dis. Reptr. 31: 130. 1947.

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[5] Bond, T. E. T. Pod spot of okra (*Hibiscus esculentus* L.) and a leaf spot of *Hibiscus rosa-sinensis* L. in Ceylon. Trop. Agr. Trin. 20: 67–70. 1943. (Abstr. in Rev. Appl. Mycol. 22: 340–341. 1943.)

[6] The assistance of C. E. Lewis, in charge, Mountain Vegetable and Fruit Station, Hendersonville, N. C., in conducting this test is gratefully acknowledged.

scribed for this species, a study was undertaken to determine the nature of the deficiencies of the *Euonymous strain.*

Ten cultures isolated from three different tumors were studied. The basic medium used had the following composition: potassium nitrate (KNO_3), 5 gm.; magnesium sulphate ($MgSO_4 \cdot 7H_2O$), 0.2 gm.; dipotassium phosphate (K_2HPO_4), 0.2 gm.; calcium chloride ($CaCl_2$), 0.1 gm.; ferrous sulphate ($FeSO_4 \cdot 7H_2O$), trace; dextrose, 10 gm.; distilled water, 1000 ml. The reaction was adjusted to pH 7.2.

Although this basic medium did not support the growth of the *Euonymous* isolates, when it was supplemented with 0.5 per cent Difco yeast extract excellent growth of the bacteria resulted. Casein hydrolysate did not replace the essential growth factors present in the yeast extract. Vitamins of the "B" complex, which included thiamin HCl, pyridoxine, riboflavin, calcium pantothenate, nicotinic acid, biotin, para aminobenzoic acid, folic acid and B12, when combined in the basic medium, permitted excellent growth of the *Euonymous* strain. When the vitamins were tested individually, however, only calcium pantothenate was effective in allowing the initiation of growth of these organisms. Bacterial multiplication in the calcium pantothenate-containing basic medium was relatively slight when compared with that found when either yeast extract or all of the "B" group of vitamins were incorporated into the basic medium. Calcium pantothenate itself could therefore not completely replace either the yeast extract or full complement of the "B" vitamins necessary for optimum growth of these organisms. When the other "B" vitamins were added singly to the calcium pantothenate-containing basic medium, there was no appreciable increase in the amount of bacterial growth over that found in the basic medium containing calcium pantothenate alone. By combining calcium pantothenate, nicotinic acid, and biotin in the basic medium, however, excellent growth of all ten bacterial isolates was obtained. These three vitamins when used together appeared capable of replacing either wholly or in very large part the growth factors present in the yeast extract.

The vitamin requirements of this organism appear to be the same as those reported by Starr[1] for *Agrobacterium rubi* (Hildebrand) Starr & Weiss. Unlike *A. rubi*, however, the *Euonymous* isolates do not appear to require dl glutamic acid in addition to the three vitamins for growth on a mineral salts-dextrose medium. The host range of the *Euonymous* strain is apparently greater than is that of the cane-gall organism. Hildebrand[2] in discussing *A. rubi* states: "Although studies on host range are incomplete, it probably deserves mention that none of the plants thus far tested except the brambles have been found susceptible to infection." Pinckard[3]

[1] Starr, Mortimer P. The nutrition of phytopathogenic bacteria. II. The genus *Agrobacterium*. Jour. Bact. 52: 187–194. 1946.

[2] Hildebrand, E. M. Cane gall of brambles caused by *Phytomonas rubi* n. sp. Jour. Agr. Res. [U. S.] 61: 685–696. 1940.

[3] Pinckard, J. A. Physiological studies of several pathogenic bacteria that induce cell stimulation in plants. Jour. Agr. Res. [U. S.] 50: 933–952. 1935.

also failed to infect tomato, beet, oleander, and olive with the cane-gall organism.

Coleman's[4] recent studies, however, extended the known host range of *A. rubi* to include the sunflower and *Vicia faba*. Tomato, which responds with tumor formation to inoculation with the *Euonymous* isolates, was not found by Coleman to be susceptible to *A. rubi*. The *Euonymous* isolates, therefore, differ both in their host range and in their nutritional requirements from the strains of *A. rubi* that have been investigated. Comparative studies of the two organisms are not sufficiently complete, however, to determine whether these differences warrant the establishment of a new bacterial species. Since the species *Agrobacterium tumefaciens* as now constituted is composed of a large group of strains that show pronounced and consistent differences in their physiological, serological, and pathological properties, the *Euonymous* isolates described here might alternatively be regarded as constituting a strain of the crown-gall bacterium that has lost its ability to synthesize three vitamins essential for optimum growth.—The Rockefeller Institute for Medical Research, New York, New York.

[4] Coleman, L. C. Tumor induction in *Vicia faba* and other hosts by *Agrobacterium rubi* (Hildebrand) Starr & Weiss. Can. Jour. Res. (C) 28: 277–282. 1950.